EFFECTS OF DOPAMINE D3 RECEPTOR ANTAGONISM ON THE DEVELOPMENT OF BEHAVIORAL SENSITIZATION TO COCAINE

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Director of Thesis

Master's Committee _____, Chair

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Morehead State University H. D.

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** $p < .01$ level, * $p < .05$ level

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ABSTRACT

Recent research suggests that dopamine D3 receptors may play an important role in the rewarding and stimulating effects of cocaine. The purpose of the present study was to determine whether concurrent treatments with the putative selective dopamine

D3 antagonist U99194A would attenuate the development of behavioral sensitization to cocaine.

In two experiments, sixty-four male Wistar rats, that weighed between 250 – 300g received 7 daily treatments with one of the following drug combination: vehicle/vehicle, vehicle/cocaine (15mg/kg, S.C.), U99194A(0.1, or 1.0 mg/kg, I.P.)/vehicle U99194A/cocaine. Each rat was first injected S.C. with either of U99194 or vehicle and then 5 min later, each rat was injected I.P. with cocaine or vehicle. Ten min after the second injection, each rat was tested for locomotor activity in photocell activity chambers (Med-Associates) for 60 min. On day eight, all rats were tested for activity after a challenge injection of cocaine (15mg/kg). On day nine, all rats were tested for activity after a challenge injection of U99194A (0.1 or 1.0 mg/kg).

The major findings were as follows: a) rats treated with cocaine alone were significantly more active during the pretreatment phase than rats treated with only vehicle; b) repeated treatments with cocaine produced behavioral sensitization; c) neither dose of U99194A affected activity; d) U99194A did not attenuate the cocaine-induced stimulation of activity; e) repeated treatment cocaine with U99914A did not prevent the development of behavioral sensitization to cocaine, and f) rats previously treated with cocaine displayed significantly greater activity after the U99194A challenge injection compared to rats pretreated with vehicle.

Taken together, these findings suggest that although repeated cocaine treatments may result in an alteration of D3 receptors, D3 receptor stimulation is not critical to the development of behavioral sensitization to cocaine.

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CHAPTER 1

INTRODUCTION

1. Psychostimulant Drug Addiction and Behavior Sensitization

Cocaine is one of the most widely abused psychostimulant drugs (Snyder, 1996). Acutely, cocaine induces euphoria and heightened arousal (Robinson & Becker, 1986). With repeated use, however, a pattern of compulsive drug-seeking and drug-taking behaviors may occur, leading to psychological addiction (Robinson & Berridge, 1993; Robinson & Becker, 1986; Berridge & Robinson, 1995). Addicted individuals often experience profound anguish and despair over powers the drug has over them and find it extremely difficult to maintain any resolve to stop using (Robinson & Becker, 1986; Kalivas & Stewart, 1991). Research indicates that chronic use of psychomotor stimulants induces long-term alterations in the central nervous system and produces behavioral sensitization (Kalivas, Duffy, DuMars, & Skinner, 1988; Snyder, 1996).

Behavioral sensitization to cocaine refers to the finding that the motor stimulant and rewarding effects of cocaine often increase with repeated use of the drug (Robinson & Berridge, 1993). It has been speculated that the neuroadaptations mediating this sensitization phenomenon may, in part, underlie the behavioral changes produced by chronic cocaine abuse, including paranoia, craving and relapse (Berridge & Robinson, 1995). Consequently, an extensive amount of research has been directed at determining the neurochemical adaptations mediating the development and expression of behavioral sensitization (Robinson & Berridge 1993; Kalivas & Stewart, 1991).

Although cocaine and other psychostimulants influence several neurochemical systems, neuroadaptations in the mesotelecephalic system have received the most

attention as the primary mechanism mediating behavioral sensitization. In an early review of the literature, for example, Robinson and Berridge (1993) concluded that: (1) Many different addictive drugs activate a common neural system, the mesotelencephalic dopamine system; (2) Repeated administration of many addictive drugs causes the dopamine system to become hypersensitive, and this is accompanied by a gradual and incremental increase (sensitization) in the psychomotor activating and incentive motivational properties of drugs; (3) The neuroadaptations underlying sensitization are extremely persistent; and (4) The expression of sensitization is subject to conditioned stimulus control.

2. Dopamine and behavioral sensitization

Nearly all psychostimulant drugs that produce behavioral sensitization from caffeine to cocaine either directly or indirectly increase neuroactivity within the mesencephalon dopamine system. Although there are several dopamine pathways, two specific pathways appear to be important for the motor-stimulating and rewarding effects of cocaine. These are the nigrostriatal and the mesolimbic dopamine systems (Kalivas & Stewart, 1991).

The nigrostriatal dopamine system originates from cell bodies located almost exclusively on the substantia nigra pars compacta on the ventral mesencephalon and project to terminal fields of the neostriatum (Kalivas & Stewart, 1991). This system appears to mediate motor preparatory processes (Cooper, Bloom & Roth, 1982).

The mesolimbic dopamine system originates from cell bodies located on the A10 region of the ventral tegmental area of the mesencephalon (Steketee, Striplin, Murray, & Kalivas, 1990). These cells project to terminal fields in the limbic system, including the nucleus accumbens, as well as the prefrontal cortex. Drug-induced stimulation of

this system produces both locomotor-activation and reward. This system is thought to facilitate the impact of stimulus reward association and incentive motivation processes (Robinson & Berridge, 1993).

3. Dopamine receptors and behavioral sensitization to cocaine

Within the mesencephalic dopamine system, five subtypes of dopamine receptors have been discovered - D1, D2, D3, D4 and D5 subtypes (Civelli, Bunzow, & Grandy, 1993; Gingrich & Caron, 1993). Based upon similarities in molecular, biochemical, and pharmacological characteristics, these receptor subtypes have been classified into two groups; the D1-like and D2-like receptors. The D1-like receptor includes the D1 and D5 subtypes. They are found post-synaptically and stimulate adenlate cyclase enzyme activity. The D2-like receptor includes D2, D3 and D4 subtypes. These are located both pre- and post- synaptically and inhibit the adenlate cyclase enzyme (Schwartz, Giros Martres, & Scokolott, 1992). The D2 receptor family has a high affinity for antipsychotic drugs and has been shown to be negatively coupled (D2, D3 and D4) to the second messenger adenlate cyclase (Boutherenet, Souil, & Matres, 1991).

Over the past ten years, a great deal of research has been directed at determining the involvement of specific dopamine receptors in the development of behavioral sensitization to psychostimulant drugs. Most of this research, however, has focused on the overall role of the D1- or D2- like receptors rather than individual subtypes within each category because of the lack of selective compounds. That is, although a number of drugs have been developed that selectively stimulate or inhibit either D1- or D2-like receptors, only recently have compounds become available that are selective to specific receptors within a particular group.

Two strategies have been used to study the role of dopamine receptors in the development of behavioral sensitization. One strategy has been to determine whether dopamine agonists selective for a particular subtype would produce behavioral sensitization with repeated treatments. The second strategy has been to determine whether concurrent treatment with selective dopamine antagonists and a psychostimulant drug would prevent the development of behavioral sensitization.

Although this research suggests the involvement of both receptor subtypes in behavioral sensitization, the exact mechanism remains unclear. This research will be reviewed in the next two sections.

4. Dopamine agonists and behavioral sensitization

Most psychostimulant drugs of abuse result in an increased stimulation of all dopamine receptors (Wise & Bozarth, 1985). For example, cocaine indirectly increases the synaptic availability of dopamine by blocking the dopamine transmission (Kalivas & Stewart, 1991). Similarly, amphetamine invokes the release of dopamine from pre-synaptic membranes, resulting in an increased stimulation of all dopamine receptors (White, 1996; Svensson, Waters, Sonesson, & Wikstrom, 1993). Likewise, apomorphine produces behavioral sensitization by directly stimulating both D1- and D2- like receptors. As noted previously, however, dopamine agonists are available that are selective to either the D1- type or D2- type of dopamine receptor.

Although the acute effects of selective dopamine agonists differ from nonselective drugs such as cocaine, research indicates that these compounds can result in behavioral sensitization with repeated treatment. For example, the selective D1-type agonist, SKF38393, produces decreases of activity when administered acutely but results in sensitization and cross-sensitization to apomorphine after chronic administration

(Mattingly, Rowlett, & Lovell, 1993). This finding suggests that repeated stimulation of D1-type receptors may induce behavioral sensitization.

Like the D1-like receptor agonists, repeated treatment with D2-like receptor agonists also produce behavioral sensitization and cross-sensitization to psychostimulants. For example, repeated administration of the D2-like dopamine agonist bromocriptine (Hoffman & Wise, 1992) has been shown to produce behavioral sensitization. Similarly, numerous studies have observed behavioral sensitization with repeated administration of the D2-like agonist quinpirole (e.g. Szechtman, Talangbayan, & Canaran, 1993; Mattingly et al., 1993). Moreover, rats sensitized to quinpirole display cross-sensitization to apomorphine (Mattingly et al., 1993) and cocaine (Henry, Hu, & White, 1998). Thus, it appears that the development of behavioral sensitization may develop as a result of repeated stimulation of either D1- or D2- like receptors.

5. Dopamine antagonists and behavioral sensitization

Research with selective dopamine agonists indicates that behavioral sensitization may develop as a result of repeated stimulation of either D1- or D2- like receptors. Although D2-receptor stimulation may induce behavior sensitization, evidence from research with selective dopamine antagonists suggests that D2 receptor stimulation may not be necessary for the development of behavior sensitization. For example, although concurrent treatment with the D2-like DA antagonist sulpiride blocks the acute effect of apomorphine, it does not block the development of sensitization to apomorphine (Mattingly, Rowlett, Graff, & Hatton, 1991). Similarly, the development of behavioral sensitization to amphetamine is not affected by concurrent treatment with the D2–type antagonists pimozide, metoclopromide, or RO-22-2586 (Drew & Glick,

1990; Stewart & Vezina, 1989). In contrast, concurrent treatment with the D1- like DA antagonist SCH23390 prevents the development of behavioral sensitization to both apomorphine (Mattingly et al., 1991) and amphetamine (Vezina & Stewart, 1989). Moreover, evidence indicates that concurrent treatment with SCH23390 also blocks the development of behavioral sensitization to the D2-like DA agonist quinpirole (Mattingly et al., 1993), and bromocriptine (Hoffman & Wise, 1992). Taken together with the DA agonist results, these findings suggest that although D2 receptor stimulation may contribute to the development of behavioral sensitization to apomorphine and amphetamine, D1 receptor stimulation is essential (Mattingly et al. 1991, 1993).

It has generally been assumed that the development of behavioral sensitization to different drugs is mediated by a common neurochemical mechanism (Robinson & Berridge, 1993; Kalivas & Stewart, 1991). Based upon research with amphetamine and apomorphine, this mechanism appeared to be related to stimulation of D1 receptors. However, recent research with cocaine questions this assumption. For example, although concurrent treatment with the D1-like DA antagonist SCH23390 prevents the development of behavioral sensitization to apomorphine (Mattingly et al., 1993) and amphetamine (Vezina & Stewart, 1989), SCH23390 treatments do not block the development of behavioral sensitization to cocaine (Mattingly, Hart, Lim, & Perkins, 1994; White, Joshi, Koeltzow, & Hu, 1998). This finding suggests that unlike apomorphine and amphetamine, stimulation of DA D1-like receptor is not necessary to the development of behavioral sensitization to cocaine.

Although SCH23390 treatment does not prevent cocaine-induced behavioral sensitization, concurrent treatment with the relatively non-selective dopamine

antagonist haloperidol does attenuate the development of behavioral sensitization to cocaine (Mattingly, Rowlett, Ellison, & Rase, 1996). Whether this haloperidol-induced attenuation of cocaine sensitization is due to blocking different DA receptors or a combination of DA receptors is currently unknown.

6. Dopamine D3 receptor and behavioral sensitization

As noted previously, three individual dopamine receptors (D2, D3, D4) have been classified as D2-like receptors. Until recently, little was known about the behavioral functions of these individual receptors because of the lack of highly selective drugs. Recently, however, several compounds have become available that appear to be relatively selective for dopamine D3 receptors (Sokoloff, Giros, Martres, Bouthentet, & Schwartz, 1990). Based upon research with these compounds, the D3 receptor has received a great deal of attention as a possible target for the treatment of cocaine abuse.

Unlike D2 receptors, D3 receptors appear to be preferentially located in limbic regions of the brain closely associated locomotor activation and reward (Levesque, Diaz, Pilon, & Martres, 1992), and the number of binding sites for D3 receptors are significantly elevated in cocaine overdose victims (Staley & Mash, 1996). In addition, 7-OH-DPAT, a putative selective agonist for D3 receptors has been reported to: a) attenuate cocaine self-administration in rats at doses not self-administrated (Caine & Coob, 1993; Nader & Mash, 1996); b) substitute for cocaine, amphetamine, and apomorphine in drug discrimination tasks (Khroyan, Fuch & Beck, 1999; Depoortere, Perrault & Sanger, 1999) and c) attenuate the incentive motivational properties of amphetamine, cocaine, and morphine as measured by conditioned place preference tests (Khroyan et. al.,1995; 1998, 1999; DeFonseca, Martin, Caine, & Nanarro, 1995).

Based upon these findings, it has been suggested that D3 receptors may be involved in the development and maintenance of drug craving.

Dopamine D3 receptors have also been related to the development of craving as measured by the development of behavioral sensitization. For example, although administration of 7-OH-DPAT decreases activity when administrated acutely, the repeated administration high doses of 7-OH-DPAT produces behavioral sensitization (Mattingly, Fields, Langfels, Rowlett, & Robinet, 1996). However, unlike other dopamine D2-type agonist, repeated treatments with 7-OH-DPAT does not increase basal dopamine synthesis, which suggests that autoreceptor sensitivity is not affected by repeated 7-OH-DPAT treatments (Svensson, Carlsson, & Waters, 1994). In addition, like cocaine, concurrent treatments with the dopamine D1 antagonist, SCH23390, does not prevent development of behavioral sensitization to 7-OH-DPAT (Mattingly, Himmler, Bonta, & Rice, 1998). These latter findings suggest that the development of behavioral sensitization to cocaine and 7-OH-DPAT may be mediated by a common neurochemical mechanism. Consistent with this possibility, recent evidence indicates that the co-treatment of rats with a low dose of 7-OH-DPAT and cocaine enhances the development of behavioral sensitization to cocaine (Mattingly, Caudill, & Abel, in press)

7. Summary and purpose

In summary, a great deal of evidence suggests that dopamine receptor stimulation is necessary for the development of behavioral sensitization to psychostimulant drugs. Although the development of behavioral sensitization to different psychostimulant drugs has been assumed to be mediated by common neurochemical mechanisms, research with selective dopamine D1- and D2-like receptor antagonists questions this

assumption. Specifically, it has been found that the development of behavioral sensitization to cocaine unlike apomorphine and amphetamine, is not prevented by concurrent treatments with either D1- or D2-type antagonists. Recent research suggests that dopamine D3 receptors may play an important role in cocaine abuse and the development of behavioral sensitization to cocaine. Moreover, it has been found that low doses of the selective dopamine D3 agonist 7-OH-DPAT enhance the development of behavioral sensitization to cocaine.

The major objective of the present research, therefore, was to further study the role of D3 receptors in the development of behavioral sensitization to cocaine by determining whether the co-administration the putative selective dopamine D3 receptor antagonist, U99194A, with cocaine would block the development of cocaine-induced behavioral sensitization.

CHAPTER 2

METHODS

Subjects

Sixty-four male Wistar albino rats (Harlan, Spraque Dawley, Indianapolis, IN) served as subjects in two experiments. The rats weighed between 258-297g prior to testing. They were housed individually in standard wire-mesh cages in a temperature-controlled colony room with a 12 hr light-dark cycle. All testing was conducted during the light phase of the cycle. The rats were housed in the colony room for at least one week prior to the beginning of the experiment. Food and water were available continuously.

Apparatus

In both experiments, activity was measured in four open field chambers (Med-Associates model OFA-163, see Figure 1), and each chamber was located in an individual sound attenuated experimental cubicle. These chambers were 41 x 41 cm., and had 16 x 16 array of infrared photocell beams placed 2.5cm above the floor and another 16 photobeams and detectors located 10 cm above the floor. A clear cylindrical acrylic chamber was positioned inside the outer square chamber. Output from each individual photocell array was connected to a Gateway-2000 (P5-75) Microcomputer through a Med-Associates interface, located in an adjacent room. Using Med-Associates software, distance traveled (cm), number of rears, and stereotypy counts (small movements) were recorded at 15-min intervals.

Drugs

In both experiments, cocaine hydrochloride (N.I.D.A.) and U99194A

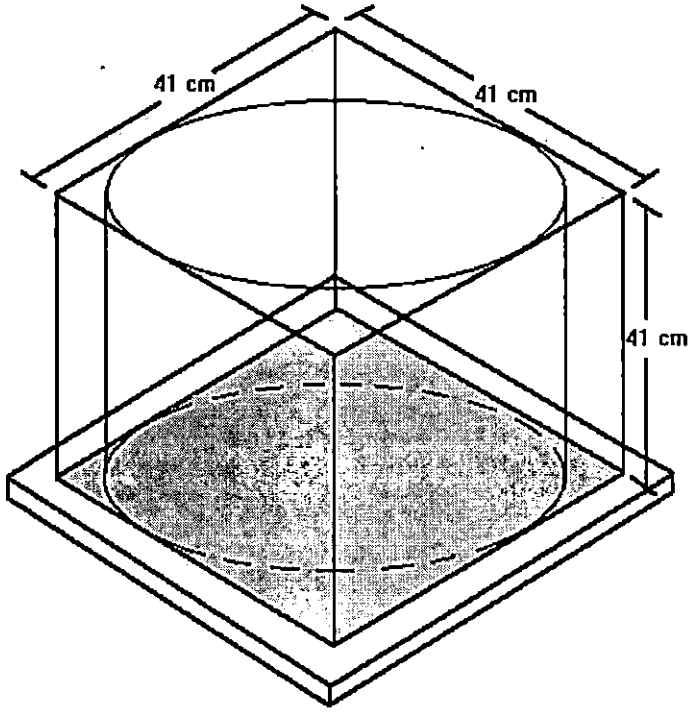

Figure 1: Med-Associates locomotor activity testing chambers

(Research Biomedicals) were dissolved in distilled water and injected in a volume of 1 ml/kg. Cocaine was injected I.P. and U99194A was injected S.C. All drug dosages were calculated based upon salt weight of the drug. Vehicle injections were given using the same route and volume as the corresponding drug injection.

Design and Procedure

The design of the experiment 1 was a 2 (Cocaine dose: 0 or 15mg/kg) x 2 (U99194A dose: 0 or 0.10mg/kg) factorial design (see Table 1). All rats were randomly assigned and to one of the four pretreatment groups. The counterbalancing Procedure is depicted in Appendix B.

Table 1: Experimental Design:

Pretreatment groups (2 x 2 Factorial Design)

	Second injection (I.P.)	
	COCAINE	VEHICLE
First injection (S.C.)		
U99194A	U-C (N=8)	U-V (N=8)
VEHICLE	V-C (N=8)	V-V (N=8)

Experiment 1 was conducted in two phases: a pretreatment phase, followed by challenge tests. During the pretreatment phase, the rats were first injected S.C. with vehicle or U99194A (0.10 mg/kg) followed 10 min later by an injection I.P. with either vehicle or cocaine (15mg/kg). Five min after the cocaine injection, the rats were put into the activity chamber for 60 min and tested for locomotor activity. This pre-treatment phase was repeated for seven days.

The challenge tests were conducted on two consecutive days beginning twenty-four hours after the pre-treatment phase. The first test was a cocaine challenge test to test for behavioral sensitization. The second one was a U99194A challenge test. The procedures were the same as during the pre-treatment phase of the experiment except the first injection was vehicle and the second injection was either cocaine (15mg/kg) or U99194A (0.10mg/kg).

In experiment 2, the design was the same as experiment 1: 2 (Cocaine dose 0 or 15mg/kg) X 2 (U99194A dose 0 or 1.0 mg/kg) factorial design. The procedure was exactly the same as experiment 1, but the dose of U99194A was 1.0mg/kg.

Data Analysis

In both experiments, significant differences among the groups in mean distance traveled, rears, and stereotypy during the pretreatment phase were analyzed using mixed four factor analyses of variance (ANOVAs) using drug treatment conditions as between factors and test session, and blocks of 15 min within sessions as repeated measures. Significant interactions were analyzed with additional ANOVAs performed on individual session and/or block data, followed by Newman-Keuls post hoc tests. Mean distance traveled, rears, and stereotypic counts of the groups on the challenge tests were analyzed using mixed three factor ANOVAs.

CHAPTER 3

RESULTS OF EXPERIMENT 1

Pretreatment Sessions: Day 1-7

Distance Traveled:

The mean distance traveled (in m) for each of the four pretreatment groups across the four 15 min blocks of sessions 1,4, and 7 is presented in Figure 2. A summary of the ANOVA performed on all seven sessions is presented in Table 2. As may be seen in Figure 2, repeated treatment with cocaine (15mg/kg) produced a progressive increase in distance traveled across sessions, cocaine effect: $F(1,28) = 210.91$, $p < .001$; Cocaine x Session interaction, $F(6,168) = 16.52$, $p < .001$; but a decrease across blocks within each session, Cocaine x Block interaction: $F(3,84) = 126.94$, $p<0.001$. The cocaine-induced increase in activity across session was greatest on the early blocks of each session, Cocaine x Session x Block interaction: $F(18,504) = 8.87$, $p < .001$. Repeated treatments with U99194A (0.1mg/kg) did not significantly affect distance traveled across the sessions or blocks within each session, U99194A effect: $F(1,28) = 2.23$, $p = 0.147$; U99194A x Session interaction: $F(6,168) < 1.00$; U99194A x Block interaction: $F(3,84) = 1.39$, $p = 0.252$; U99194A x Session x Block interaction: $F(18,504) < 1.00$. U99194A did not significantly affect cocaine-induced changes of distance traveled.

Rears:

The mean number of rears for the four treatment groups across the four 15 min blocks of sessions 1, 4, and 7 is presented in Figure 3. A summary of the ANOVA performed on the seven sessions is presented in Table 3. Overall, cocaine treatments significantly increased rearing behaviors during the pretreatment sessions, cocaine

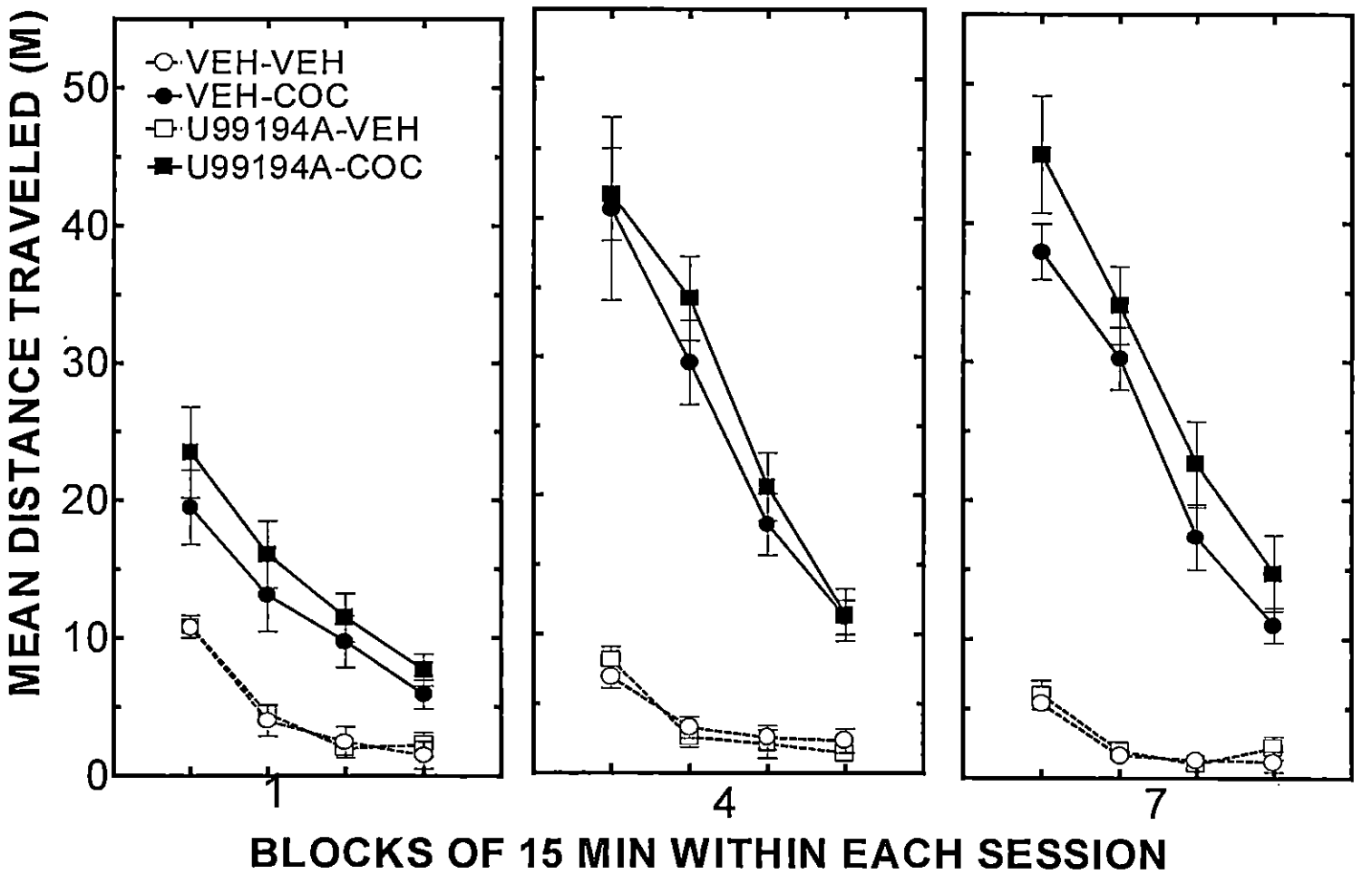

Figure 2.Mean distance traveled (± SEM) across blocks of 15 min on sessions1,4, and 7 for rats treated 15 min before each session with either vehicle (VEH), or 15 mg/kg cocaine (COC) and 5 min before each session with either vehicle (VEH) or 0.10 mg/kg U99194A

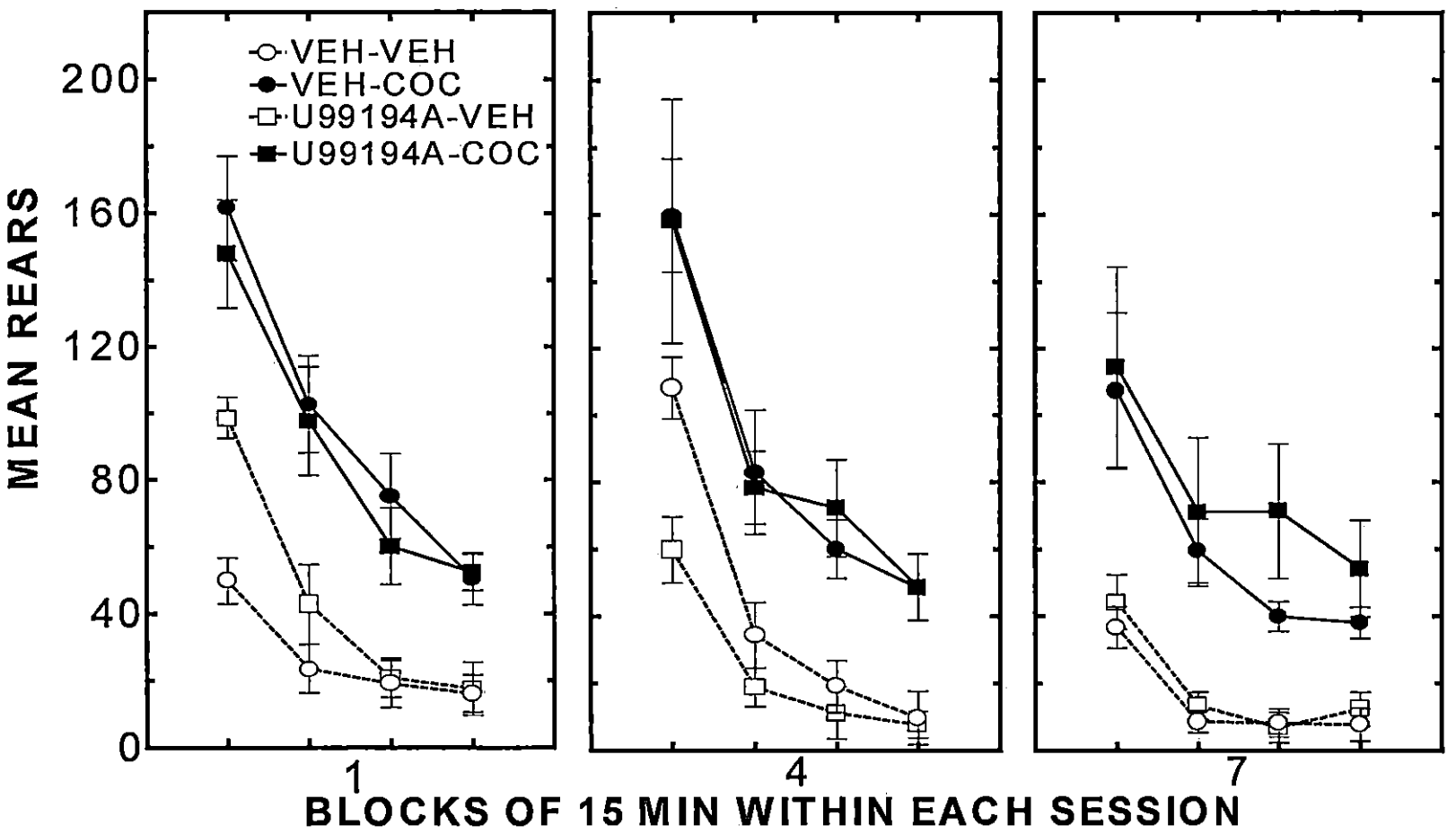

Figure 3. Mean number of rears (± SEM) across blocks of 15 min on sessions1,4, and 7 for rats treated 15 min before each session with either vehicle (VEH), or 15 mg/kg cocaine (COC) and 5 min before each session with either vehicle (VEH) or 0.10 mg/kg U99194A

effect: $F(1,28) = 52.10$, $p < .001$. However, this cocaine induced increase in reading decreased across both session and blocks, Cocaine x Block interaction: $F(3,84) = 9.02$, $p<.001$; Cocaine x Session x Block: $F=2.01$, $p<0.01$. U99194A did not significantly affect rearing behavior, U99194A effect: $F(1,28) < 1.00$; U99194A x Block: $F(3,84) <1.00$. U99194A x Session interaction: $F(6,168) < 1.00$. More important, U99194A did not significantly affect cocaine-induced changes in rearing behavior, Cocaine x U99194A interaction: $F(1, 28) < 1.00$.

Stereotypy:

Figure 4 displays the mean stereotypic counts for the four pretreatment groups during sessions 1,4, and 7. A summary of the ANOVA performed on the seven sessions is presented in Table 4. As may be seen in Figure 4, similar to the distance traveled and rearing results, cocaine produced an overall increase in stereotypic counts, but a decrease across blocks within each session during the pretreatment sessions, cocaine effect: $F(1,28) = 446.71$, $p< .001$; Cocaine x Block interaction: $F(3,84) = 19.23$, $p< .001$. Stereotypic counts remained stable across sessions for cocaine treated rats, but declined across sessions for rats not treated with cocaine, Cocaine x Session effects: $F(6,168) = 8.89$, $p < .001$. U99194A did not significantly affect the stereotypy, and there was no significant interactions between U99194A and cocaine, U99194A and session, U99194A and block, U99194A effect: $F(1,28) <1.0$; U99194A x Cocaine interaction: $F(1,28) < 1.00$; U99194A x Session interaction: $F(6,168) = 2.03$, $p> .05$; U99194A x Blocks interaction: $F(3,84) <1.00$; U99194A x Session x Blocks interaction: $F(18,504) <1.00$. U99194A did not significantly affect cocaine-induced increases of stereotypy.

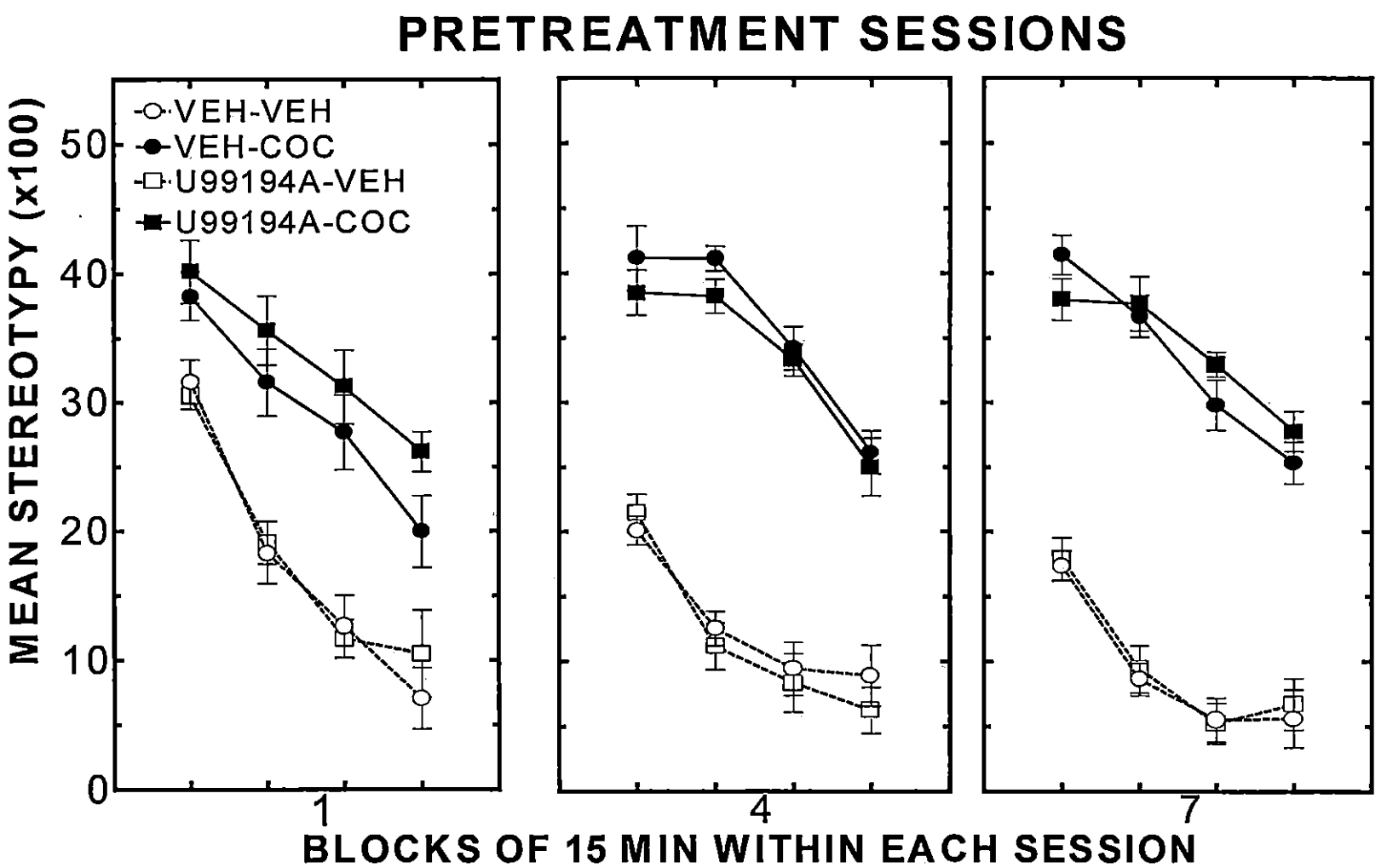

Figure 4. Mean stereotypic count (± SEM) across blocks of 15 min on sessions1,4, and 7 for rats treated 15 min before each session with either vehicle (VEH), or 15 mg/kg cocaine (COC) and 5 min before each session with either vehicle (VEH) or 0.10 mg/kg U99194A

Sensitization Test Session 8: Cocaine Challenge Test

Distance traveled:

Figure 5 displays the mean distance traveled for the four pretreatment groups after a 15mg/kg dose of cocaine. A summary of the ANOVA performed on this data is presented in Table 5. Overall, as may be seen in the left panel, prior treatment with cocaine with or without U99194A significantly increased subsequent behavioral sensitivity to the activating effects of the cocaine injection, cocaine effect: $F(1,28) = 21.79$. $p < .001$. More important, prior treatments with U99194A (0.1mg/kg) alone did not significantly affect subsequent sensitivity to cocaine, and the concurrent treatments of U99194A and cocaine did not block the development of sensitization to cocaine, U99194A effect: $F < 1.00$; U99194A x Cocaine interaction: $F < 1.00$. However, as shown in the right panel, although all groups tended to decrease activity across blocks, block effect: $F(3,84) = 149.05$, $p < .001$, this decrease was greater for rats previously treated with cocaine, Cocaine x Block interaction: $F(3,84) = 9.86$, $p < .001$. There were no interactions among U99194A, cocaine and block factors, U99194A x Cocaine x Block: $F < 1.0$.

Rears:

Figure 6 displays the mean number of rears for the four pretreatment groups after 15mg/kg dose of cocaine. A summary of the ANOVA performed on this data is presented in Table 6. As may be seem in the left panel, pretreatment with neither cocaine nor U99194A significantly affected subsequent sensitivity to a challenge injection of cocaine, cocaine effect: $F(1,28) < 1.0$; U99194A effect: $F(1,28) > 1.0$, U99194A x Cocaine interaction, $F < 1.0$. All groups appeared to decrease sensitivity

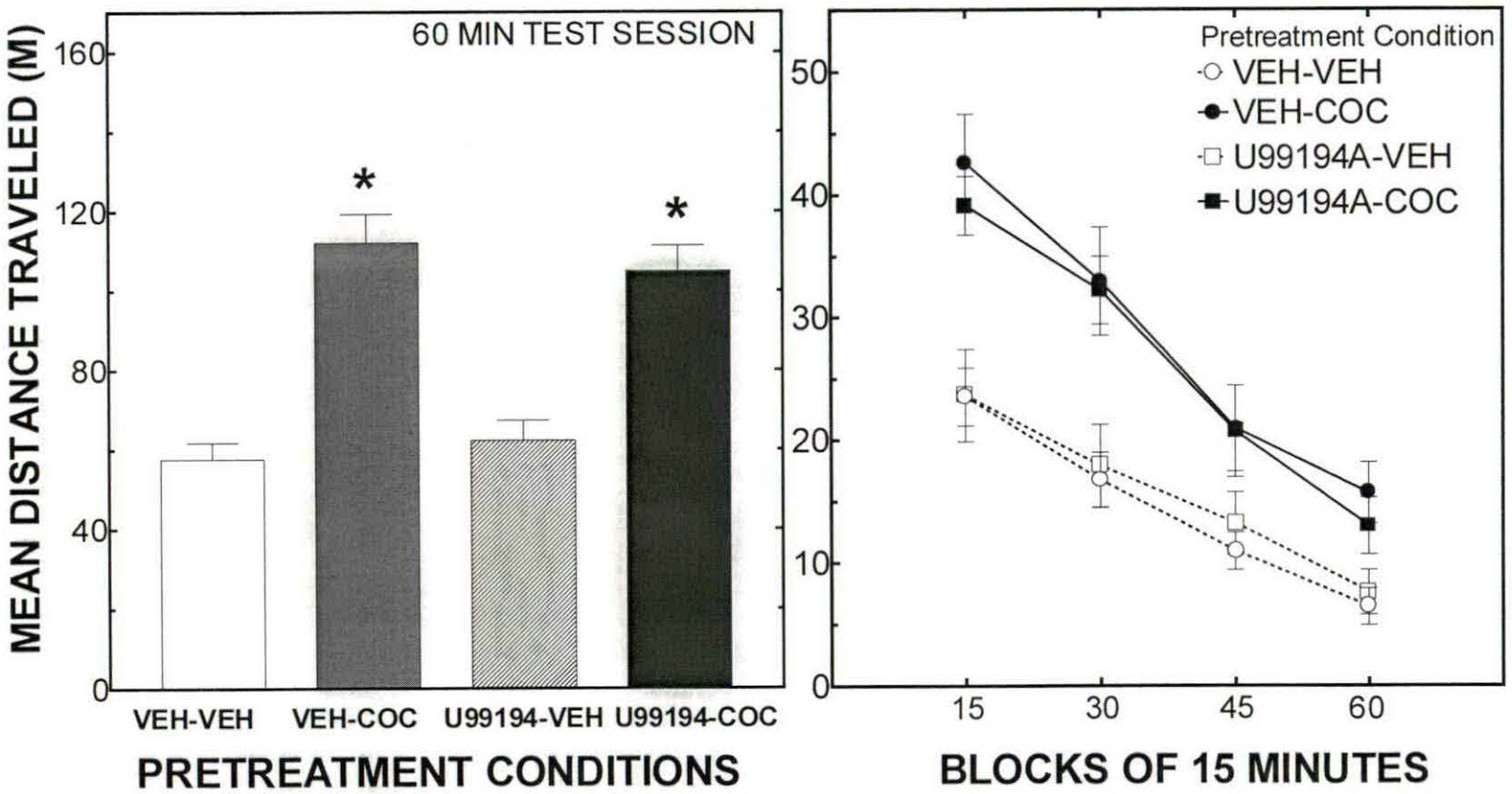

Figure 5. Mean distance traveled (± SEM) after a cocaine challenge injection (session 8) for rats previously treated chronically with either vehicle (VEH) or cocaine (15 mg/kg COC) in combination with either vehicle (VEH) or U99194A (0.10 mg/kg). The left panel represents the total session activity and the right panel presents the same data as a function of four 15 min blocks within the session

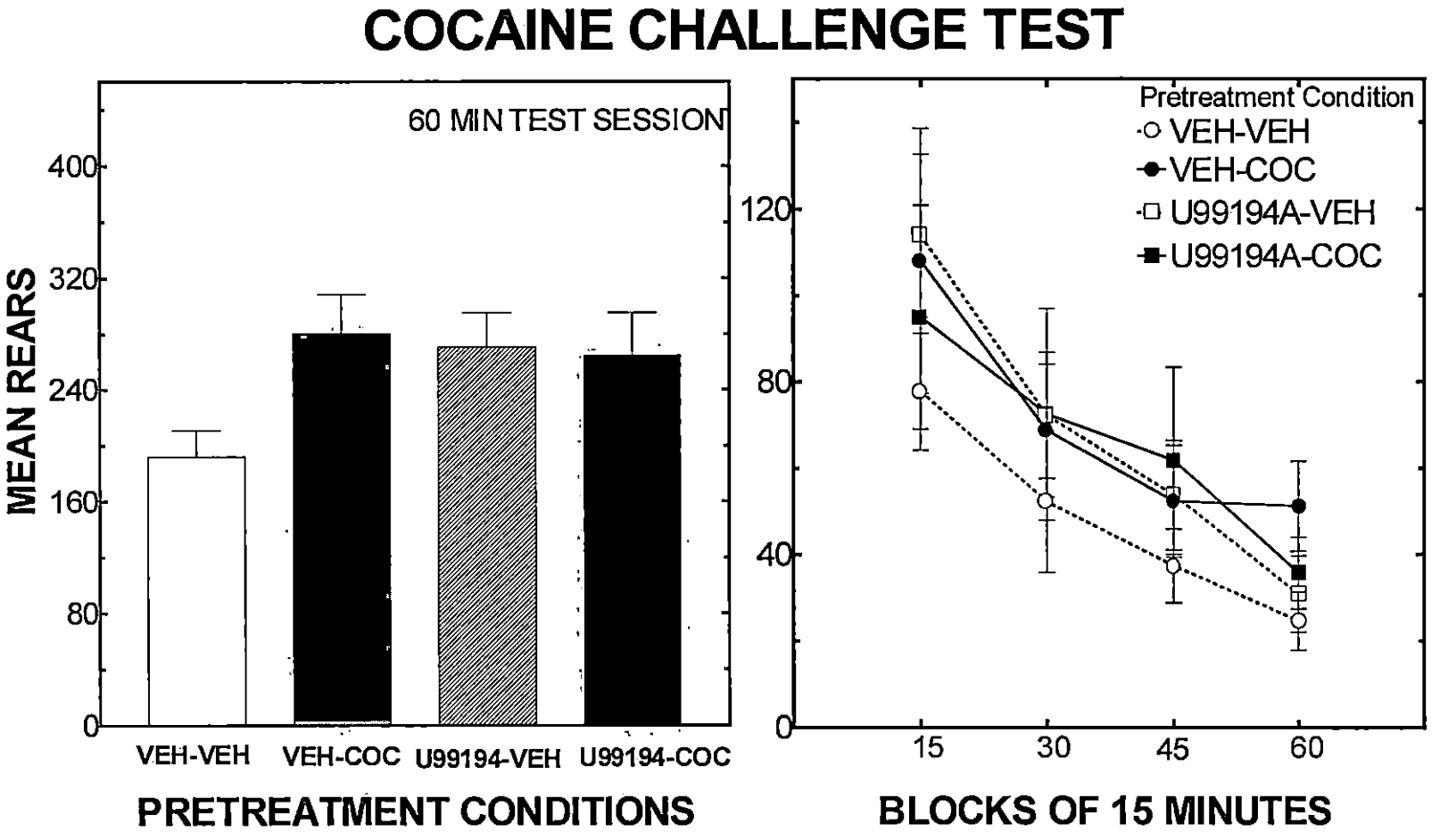

Figure 6. Mean number of rears (± SEM) after a cocaine challenge injection (session 8) for rats previously treated chronically with either vehicle (VEH) or cocaine (15 mg/kg COC) in combination with either vehicle (VEH) or U99194A (0.10 mg/kg). The left panel represents the total session activity and the right panel presents the same data as a function of four 15 min blocks within the session

across blocks, block effect, $F(3,48) = 25.70$, $p<.001$, but there were no significant interactions between them.

Stereotypy:

Figure 7 displays the mean number of stereotypic counts for the four pretreatment groups after a 15mg/kg dose of cocaine. A summary of the ANOVA performed on this data is presented in Table 7. As may be seen in the left panel, pretreatment with neither cocaine nor U99194A significantly affected subsequent sensitivity to a challenge injection of cocaine, cocaine effect: $F(1,28) = 1.70$, $p=.203$; U99194A effect: $F(1,28) < 1.00$; U99194A x Cocaine interaction: $F(1,28) < 1.00$, the changes did not obviously vary across the pretreatment groups, U99194A x Block: $F(3,84) < 1.0$; Cocaine x Block interaction: $F(3,84) = 2.82$, $p = .044$; U99194A x Cocaine x Block interaction $F(3, 84) = 1.80$, $p >= .153$. Thus, pretreatment with cocaine did not result in behavioral sensitization using stereotypic counts as a behavioral measure.

Sensitization Test Session 9: U99194A Challenge Test

Distance Traveled:

Figure 8 displays the mean distance traveled for the four pretreatment groups after a 0.10 mg/kg dose of U99194A. A summary of the ANOVA performed on this data is presented in Table 8. Overall, as may be seen in the left panel, prior treatment with cocaine significantly increased subsequent behavioral sensitivity to the activating effects of the U99194A challenge injection, cocaine effect: $F(1,28) = 19.53$, $p<.001$. This increased response to the U99194A challenge injection in cocaine pretreated rats, decreased across blocks, block effect: $F(3,83)=80.76$, $p<.001$; Cocaine x Block interaction: $F(3,84)=7.42$, $p<.001$. Prior treatments with U99194A alone did not

COCAINE CHALLENGE TEST

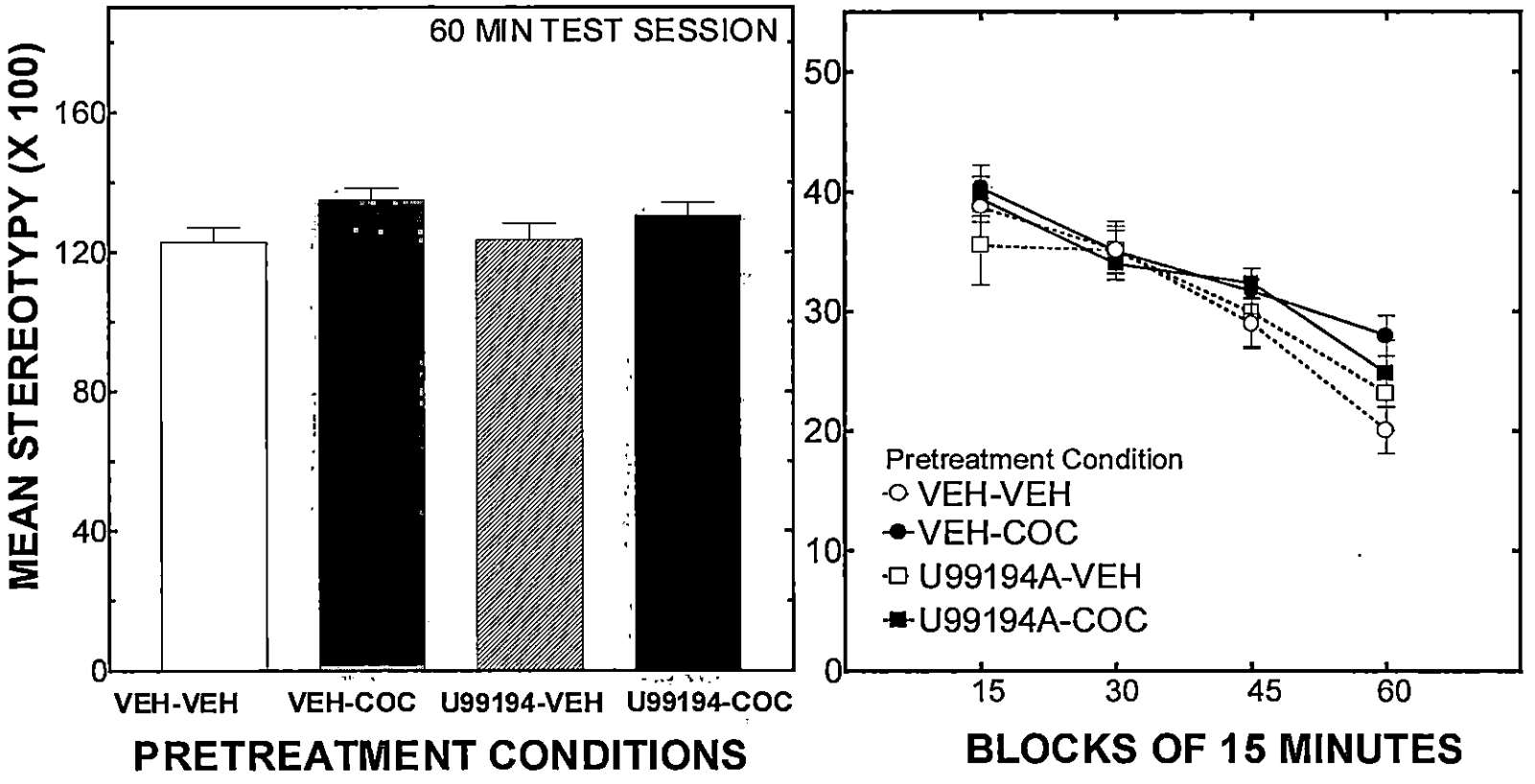

Figure 7. Mean stereotypic counts (± SEM) after a cocaine challenge injection (session 8) for rats previously treated chronically with either vehicle (VEH) or cocaine (15 mg/kg COC) in combination with either vehicle (VEH) or U99194A (0.10 mg/kg). The left panel represents the total session activity and the right panel presents the same data as a function of four 15 min blocks within the session

U99194 CHALLENGE TEST

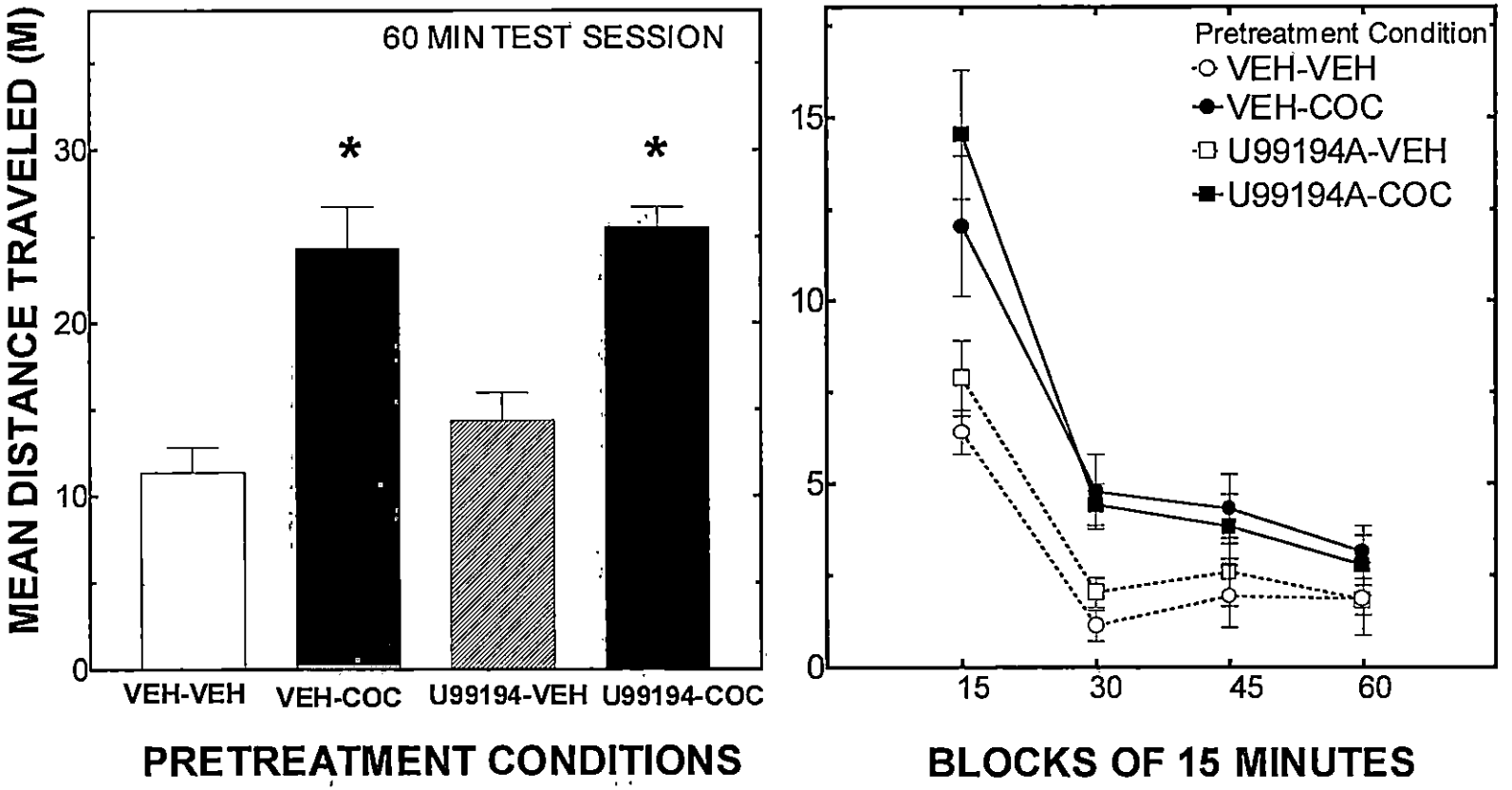

Figure 8 Mean distance of traveled (± SEM) after a U99194A challenge injection (session 8) for rats previously treated chronically with either vehicle (VEH) or cocaine (15 mg/kg COC) in combination with either vehicle (VEH) or U99194A (0.10 mg/kg). The left panel represents the total session activity and the right panel presents the same data as a function of four 15 min blocks within the session

significantly affect subsequent sensitivity to U99194A. U99194A effect: $F(1,28) < 1.00$; U99194A x Cocaine interaction: $F(3,84) < 1.00$.

Rears:

Figure 9 displays the mean number of rears for the four treatment groups after 0.10 mg/kg dose of U99194A. A summary of the ANOVA performed on this data is presented in Table 9. As may be seen in the left panel, pretreatment with cocaine significantly increased subsequent sensitivity to a challenge injection of U99194A, cocaine effect: $F(1,28) = 12.3$, $p<.005$. More important, this cocaine–induced increased increase was the same for rats also pretreated with vehicle or U99194A, U99194A x Cocaine interaction: $F(1,28) < 1.00$. However, as shown on the right panel, all groups appeared to decrease sensitivity across blocks, block effect: $F(3,84) = 44.11$, $p<.001$, but there were no interactions between U99194A and block, cocaine and block, U99194A, cocaine and block factors, U99194A x Block: $F(3,84) < 1.00$; Cocaine x Block: $F(3,84) = 1.34$, $p=.268$; U99194A x Cocaine x Block interaction: $F(3,84)<1.00$. U99194A pretreated alone did not significantly affect subsequent sensitivity to the U99194A challenge injection, U99194A effect: $F(1,28)<1.0$.

Stereotypy:

Figure 10 displays the mean number of stereotypic counts for the four pretreatment groups after a 0.10 mg/kg dose of U99194A. A summary of the ANOVA performed on this data is presented in Table 10. As may be seen in the left panel, pretreatment with cocaine increased the subsequent sensitivity to a challenge injection of U99194A, but pretreatment with U99194A did not significantly affect subsequent sensitivity to a challenge injection of U99194A, U99194A effect: $F(1,28)=1.15$, $p>.05$; cocaine effect: $F(1,28) = 15.43$, $p< .001$; U99194A x Cocaine interaction:

$F(1,28)<1.00$]. Stereotypic counts significantly decreased across the blocks, block effect: $F(3,84) = 65.44$, $p<.001$. There were no significant interactions between U99194A and block, cocaine and block, U99194A, cocaine and block factors, U99194A x Block interaction: $F(3,84) < 1.00$; Cocaine x Block interaction: $F(3,48) = 1.03$, $p=0.386$; U99194A x Cocaine x Block interaction: $F(3,84) < 1.00$].

U99194 CHALLENGE TEST

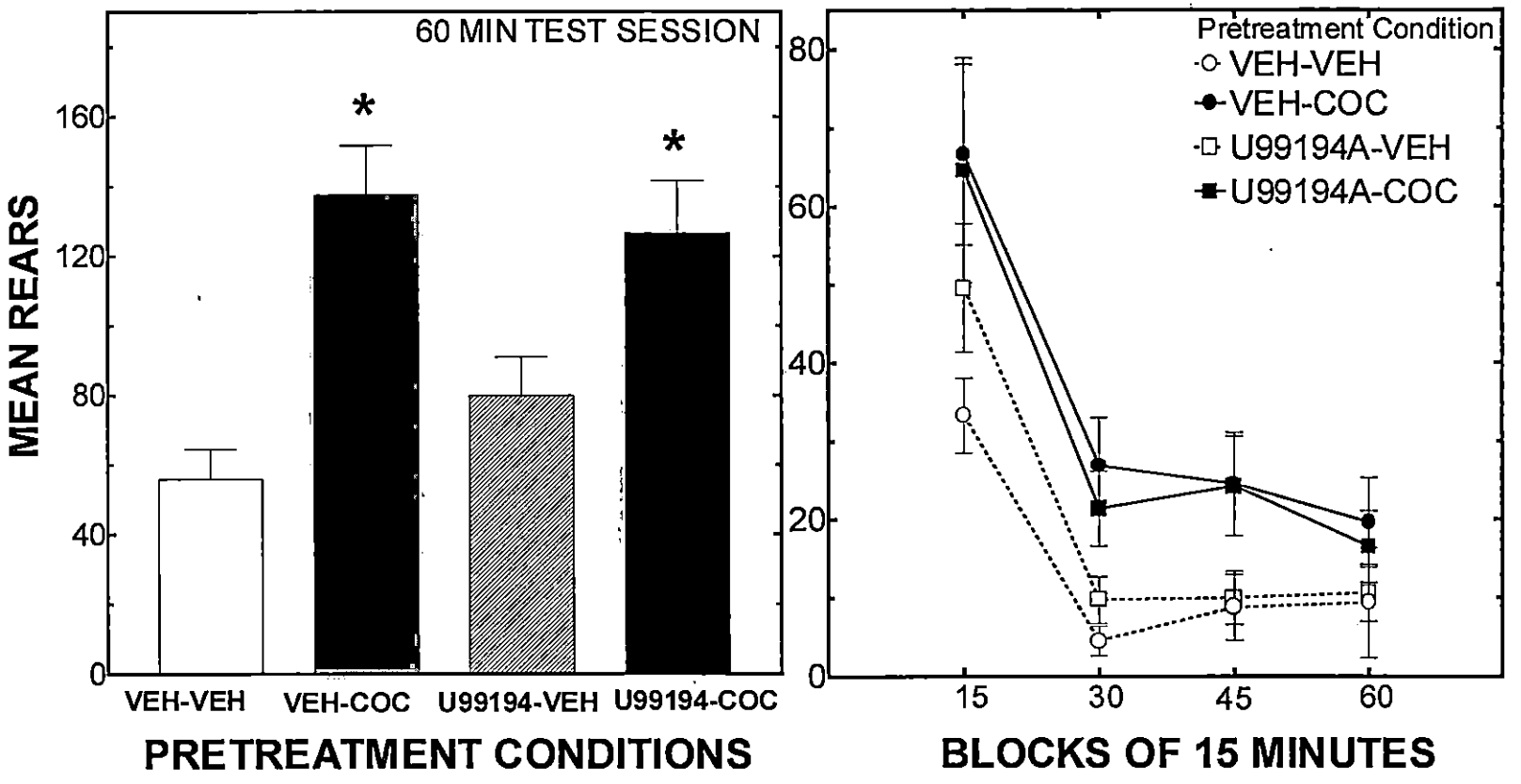

Figure 9. Mean number of rears (± SEM) after a U99194A challenge injection (session 8) for rats previously treated chronically with either vehicle (VEH) or cocaine (15 mg/kg COC) in combination with either vehicle (VEH) or U99194A (0.10 mg/kg). The left panel represents the total session activity and the right panel presents the same data as a function of four 15 min blocks within the session

U99194 CHALLENGE TEST

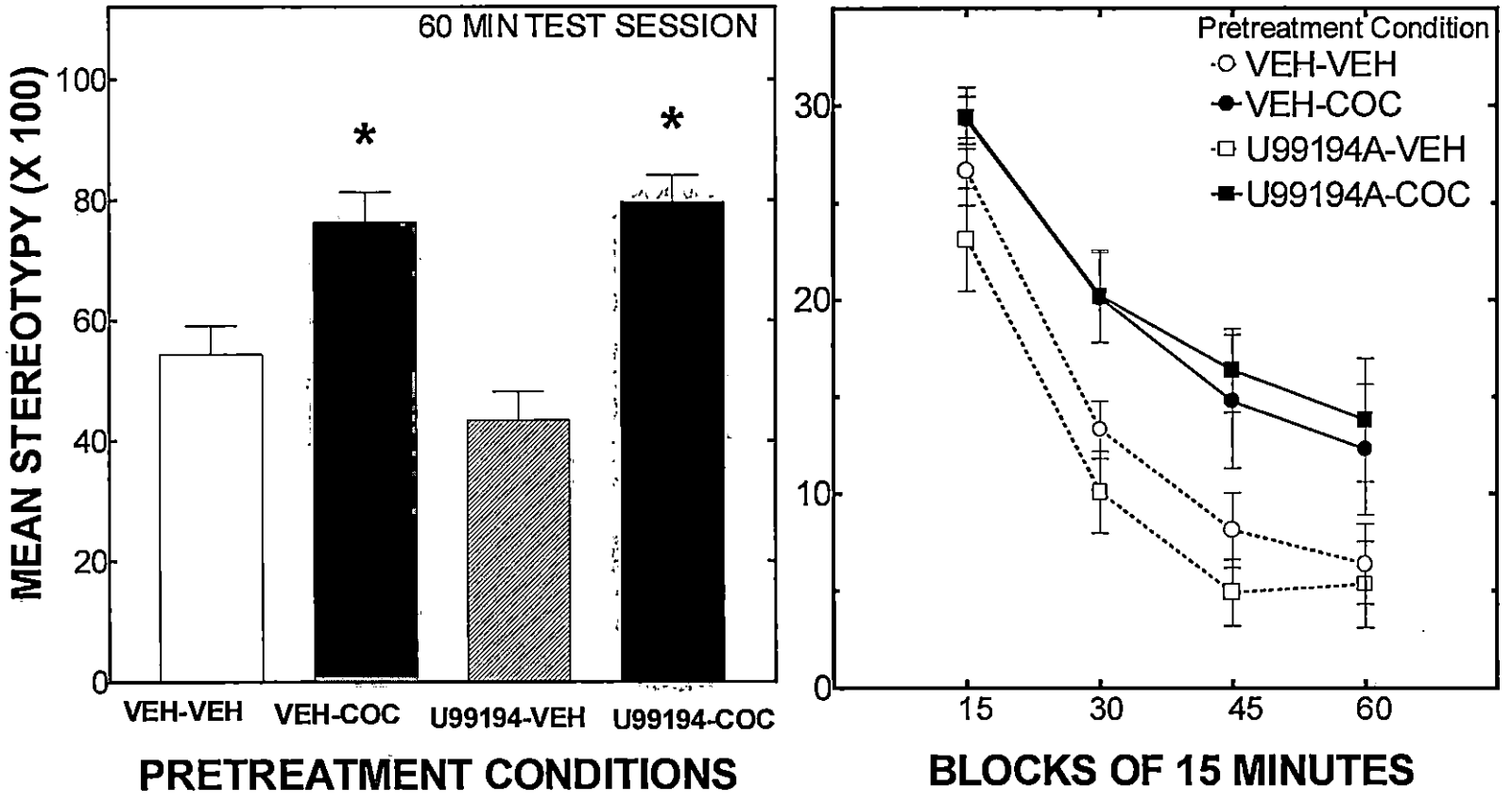

Figure 10. Mean stereotypic counts (± SEM) after a U99194A challenge injection (session 9) for rats previously treated chronically with either vehicle (VEH) or cocaine (15 mg/kg COC) in combination with either vehicle (VEH) or U99194A (1.00 mg/kg). The left panel represents the total session activity and the right panel presents the same data as a function of four 15 min blocks within the session

CHAPTER 4

RESULTS OF EXPERIMENT 2

Pretreatment Sessions- Day 1-7

Distance Traveled:

The mean distance traveled in meters for each of the four pretreatment groups across the four 15 min blocks of session 1,4, and 7 is presented in Figure11. A summary of the ANOVA performed on all seven sessions is presented in Table11. As may be seen in Figure11, repeated treatment with cocaine (15mg/kg) produced a progressive increase in distance traveled across sessions and a decrease across blocks within each session, cocaine effect: $F(1,28) = 155.61$, $p < .001$; Cocaine x Session interaction, $F(6,168) = 10.32$, $p < .001$; Cocaine x Session x Block interaction: $F(18,504) = 5.12$, $p < .001$. Repeated treatments with U99194A (1.0mg/kg) did not affect distance traveled across sessions or blocks within each session, U99194A effect: $F(1,28) = 1.84$, $p = .185$; U99194A x Session interaction: $F(6,168) < 1.0$; U99194A x Block interaction: $F(3,84) = 2.06$, $p = .111$; U99194A x Session x Block interaction: $F(18,504) < 1.0$. U99194A did not significantly affect cocaine-induced changes of distance traveled.

Rears:

The mean number of rears for the four treatment groups across the four 15 min blocks of sessions 1, 4, and 7 is presented in Figure 12. A summary of the ANOVA performed on the seven sessions is presented in Table 12. Overall, cocaine treatments significantly increased rearing behaviors during the pretreatment sessions, cocaine effect: $F(1,28) = 194.58$, $p < .001$. The effects of cocaine (15mg/kg) treatments decreased across blocks within sessions, Cocaine x Block interaction: $F(3,84) = 18.29$,

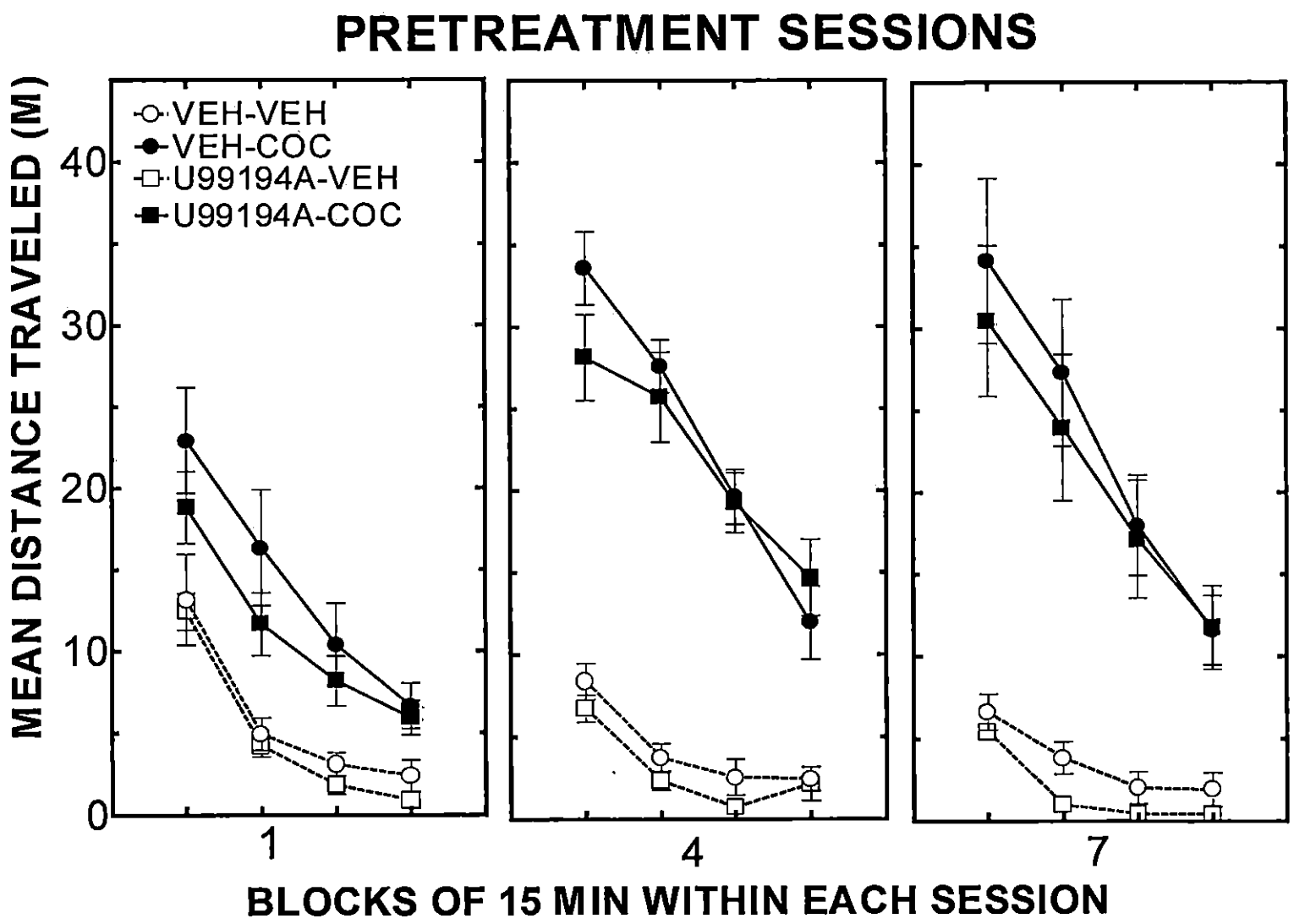

Figure 11. Mean distance traveled (± SEM) across blocks of 15 min on sessions1,4, and 7 for rats treated 15 min before each session with either vehicle (VEH), or 15 mg/kg cocaine (COC) and 5 min before each session with either vehicle (VEH) or 1.00 mg/kg U99194A

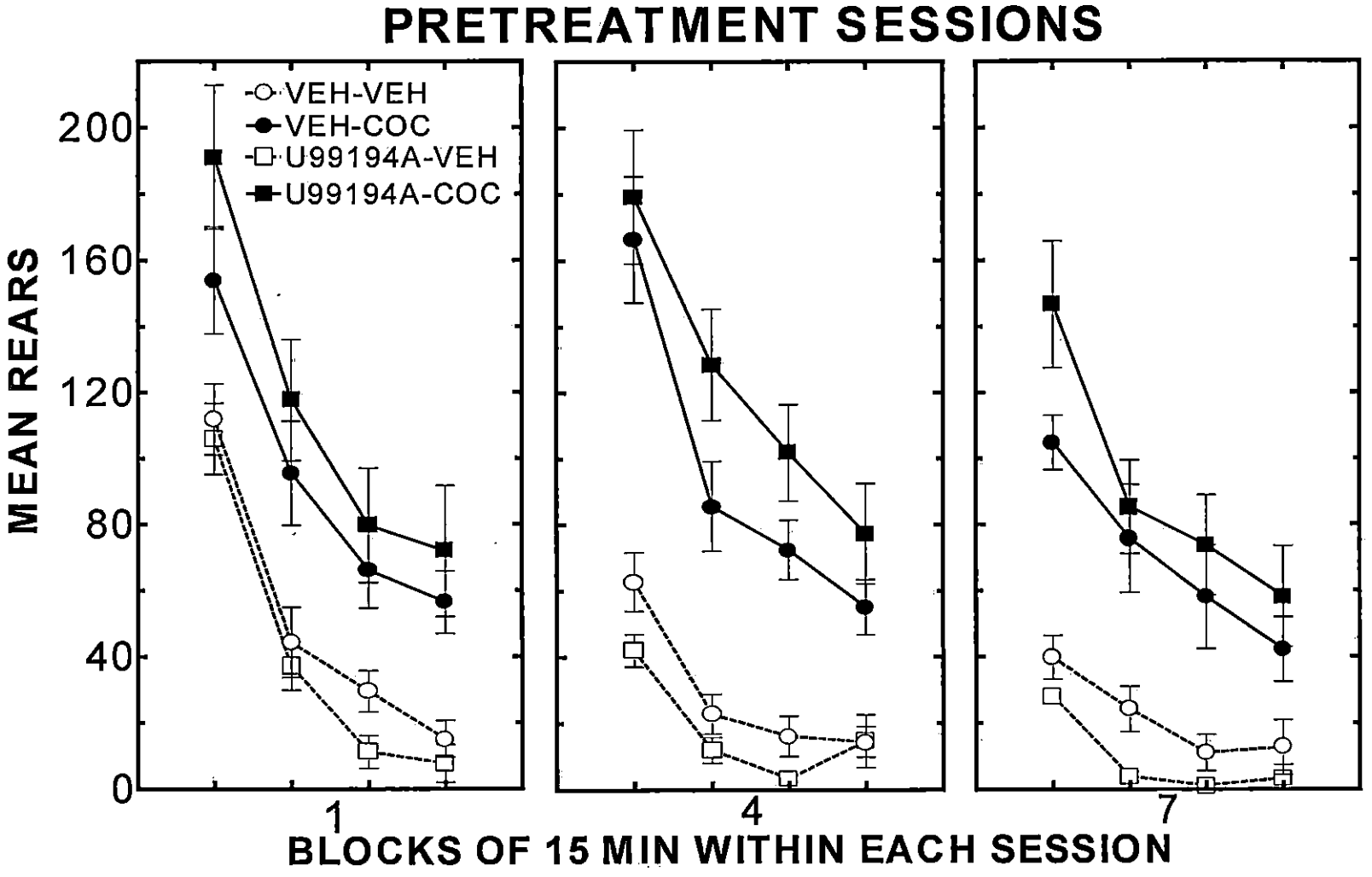

Figure 12. Mean number of rears (± SEM) across blocks of 15 min on sessions1,4, and 7 for rats treated 15 min before each session with either vehicle (VEH), or 15 mg/kg cocaine (COC) and 5 min before each session with either vehicle (VEH) or 1.00 mg/kg U99194A

p<.001, and cross sessions on some blocks, Cocaine x Session x Blocks interaction, F(18, 504)=2.09, p<.01. Overall, U99194A did not significantly affect rearing behaviors, U99194A effects: F(1,28) < 1.00, but it did appear to enhance the cocaine-induce increase in rearing behavior, U99194A x Cocaine interaction: F(1,28)=10.21, p<.01. There was no interaction between U99194A and sessions or U99194A and blocks, U99194A x Session interaction: F(6,168) < 1.00; U99194A x Block interaction: F(3,84) < 1.00.

Stereotypy:

Figure 13 displays the mean stereotypic counts for the four pretreatment groups during sessions 1,4, and 7. A summary of the ANOVA performed on the seven sessions is presented in Table 13. As may be seen in Figure 13, similar to the distance traveled and rearing results, cocaine produced an increase in stereotypic counts across sessions and a decrease across blocks within each session, cocaine effect: F(1,28) = 510.35, p< .001; Cocaine x Session effects: F(6,168) = 5.62, p < .001; Cocaine x Block interaction: F(3,84) = 14.93, p< .001. U99194A had no significant effect, and there was no significant interactions between U99194A and cocaine, U99194A and sessions, U99194A and blocks, U99194A effect: F(1,28) =2.32, p=.139; U99194A x Cocaine interaction: F(1,28) < 1.00; U99194A x Session interaction: F(6,168) < 1.00; U99194A x Block interaction: F(3,84) = 1.77, p= .159; U99194A x Session x Block interaction: F(18,504) <1.00. U99194A did not significantly affect cocaine-induced changes of stereotypic counts.

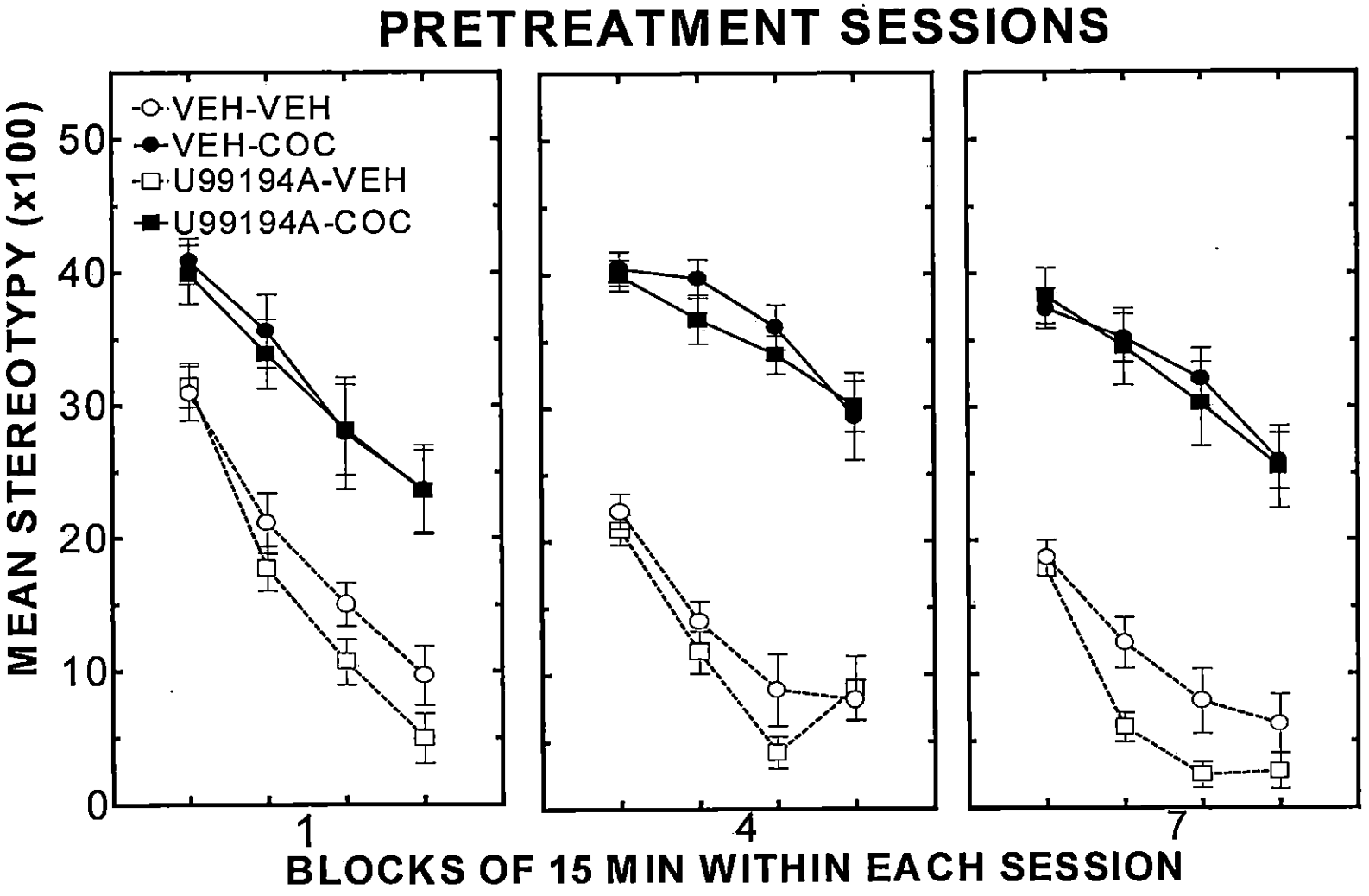

Figure 13. Mean stereotypic count (± SEM) across blocks of 15 min on sessions1,4, and 7 for rats treated 15 min before each session with either vehicle (VEH), or 15 mg/kg cocaine (COC) and 5 min before each session with either vehicle (VEH) or 1.00 mg/kg U99194A

Sensitization Test Session 8: Cocaine Challenge Test

Distance traveled:

Figure 14 displays the mean distance traveled for the four pretreatment groups after a 15mg/kg dose of cocaine. A summary of the ANOVA performed on this data is presented in Table 14. Overall, as may be seen in the left panel, prior treatment with cocaine with or without U99194A significantly increased subsequent behavioral sensitivity to the activating effects of the cocaine injection, cocaine effect: $F(1,28) = 55.44$. $p < .001$. More important, prior treatments with U99194A alone did not significantly increase subsequent sensitivity to cocaine, and the concurrent treatments of U99194A and cocaine did not block the development of sensitization to cocaine, U99194A effect: $F < 1.00$; U99194A x Cocaine interaction: $F < 1.00$. However, as show on the right panel, all groups tended to decrease activity across blocks, block effect: $F(3,84) = 76.92$, $p < .001$. The cocaine effect decreased across blocks, Cocaine x Block interaction: $F(3,84) = 31.08$, $p < .001$.

Rears:

Figure 15 displays the mean number of rears for the four pretreatment groups after 15mg/kg dose of cocaine. A summary of the ANOVA performed on this data is presented in Table 15. As may be seen in the left panel, pretreatment with cocaine significantly increased subsequent sensitivity to a challenge injection of cocaine, but U99194A did not significantly affect subsequent sensitivity to a challenge injection of cocaine, cocaine effect: $F(1,28) = 6.58$, $p = 0.016$; U99194A effect: $F(1,28) < 1.00$. More important, concurrent pretreatment with cocaine and U99194A (1.0mg/kg) did not block the development of behavioral sensitization to cocaine, U99194A x Cocaine interaction: $F < 1.0$. All groups appear to decrease sensitivity across blocks, but there

COCAINE CHALLENGE TEST

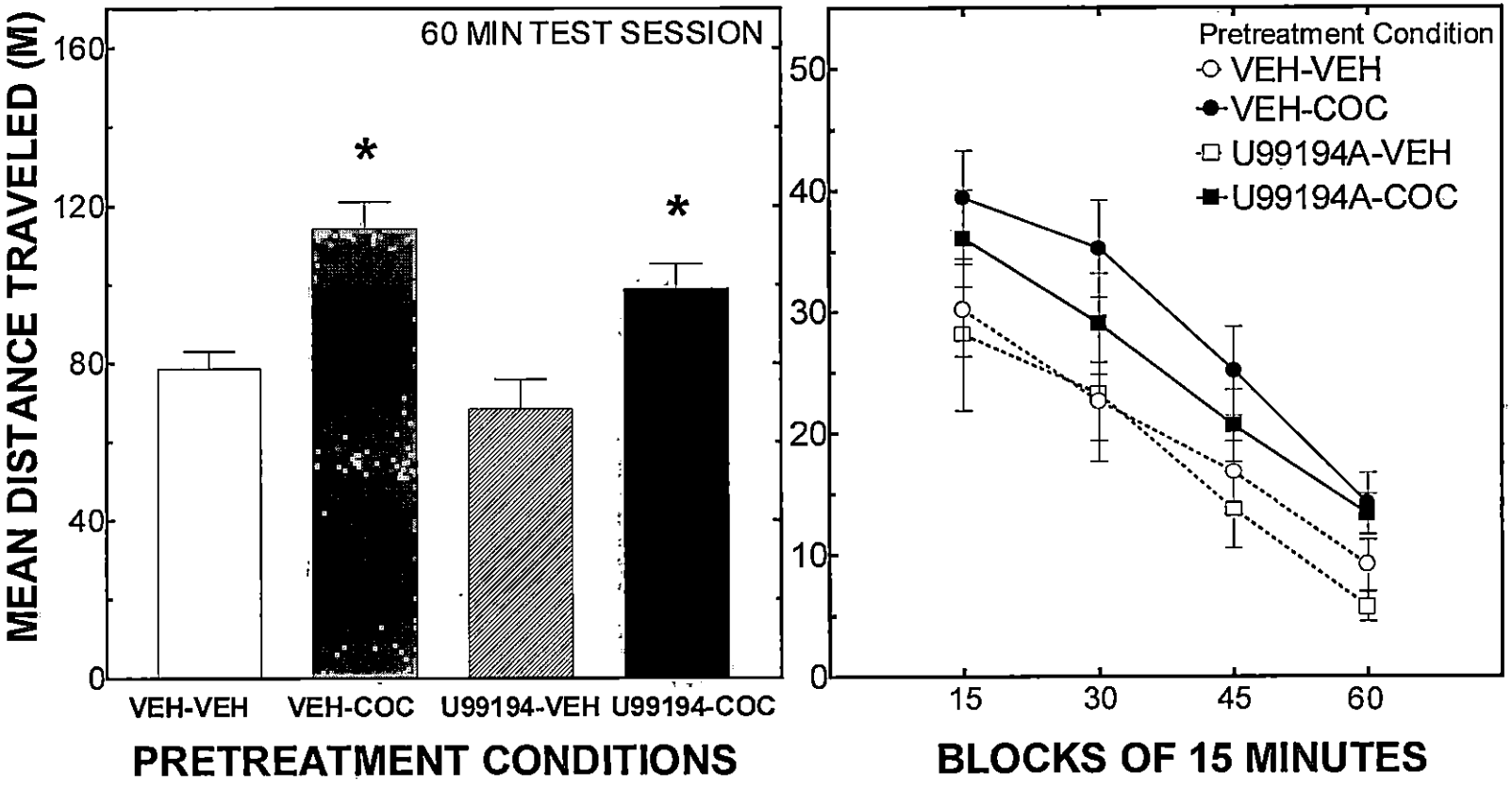

Figure 14. Mean distance traveled (± SEM) after a cocaine challenge injection (session 8) for rats previously treated chronically with either vehicle (VEH) or cocaine (15 mg/kg COC) in combination with either vehicle (VEH) or U99194A (1.00 mg/kg). The left panel represents the total session activity and the right panel presents the same data as a function of four 15 min blocks within the session

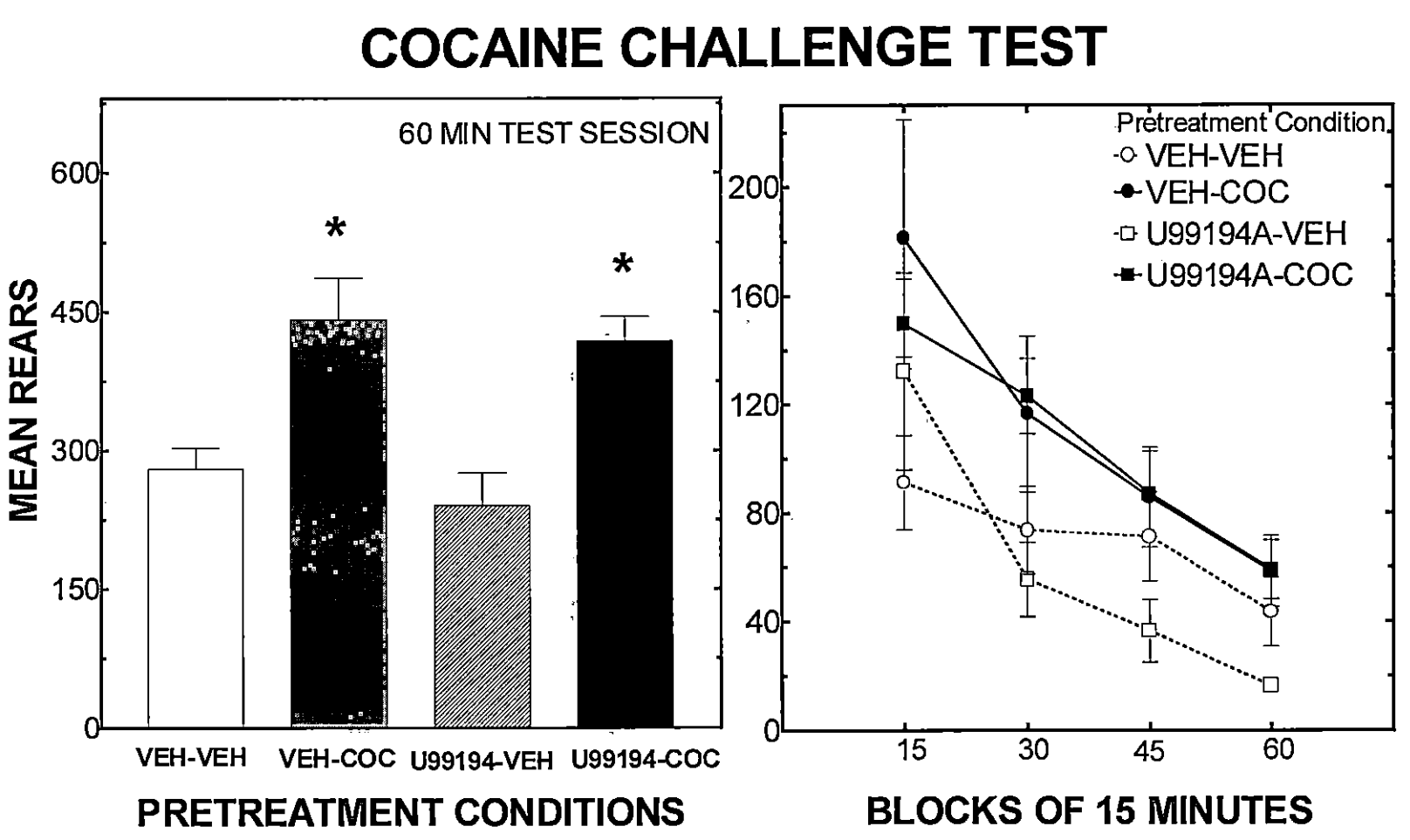

Figure 15. Mean number of rears (± SEM) after a cocaine challenge injection (session 8) for rats previously treated chronically with either vehicle (VEH) or cocaine (15 mg/kg COC) in combination with either vehicle (VEH) or U99194A (1.00 mg/kg). The left panel represents the total session activity and the right panel presents the same data as a function of four 15 min blocks within the session

were no interactions between cocaine and block factors, U99194A and block factors, block effect: $F(3,84)=33.15$, $p<.001$. Cocaine appeared to increase rears on block 1 and 2, compared with the rats in vehicle group, but U99194A appeared to decrease rears on block 3 and 4, U99194A x Cocaine x Block interaction, $F(3,84)=3.40$, $p<.05$. There was no interaction between U99194A and block factors, U99194A x Block $F(3,84)=0.47$, $p=.702$.

Stereotypy:

Figure 16 displays the mean number of stereotypic counts for the four pretreatment groups after a 15mg/kg dose of cocaine. A summary of the ANOVA performed on this data is presented in Table 16. As may be seen in the left panel, pretreatment with neither cocaine nor U99194A significantly affected subsequent sensitivity to a challenge injection of cocaine, cocaine effect: $F(1,28) <1.00$; U99194A effect: $F(1,28) < 1.36$, $p=0.253$; U99194A x Cocaine interaction: $F(1,28) =2.41$, $p=0.132$; U99194A x Block: $F(3,84) > 1.00$. Compared with vehicle groups, the stereotypic counts of rats pretreated with cocaine appeared to decrease less across blocks, Cocaine x Block interaction: $F(3,84) = 6.53$, $p < .001$; U99194A x Cocaine x Block interaction $F(3, 84) >1.00$. Thus, pretreatment with cocaine did not result in behavioral sensitization with stereotypic counts as a behavioral measure.

Sensitization Test Session 9: U99194A Challenge Test

Distance Traveled:

Figure 17 displays the mean distance traveled for the four pretreatment groups after a 1.0mg/kg dose of U99194A. A summary of the ANOVA performed on this data is presented in Table 17. Overall, as may be seen in the left panel, prior treatment with cocaine significantly increased subsequent behavioral sensitivity to the activating

COCAINE CHALLENGE TEST

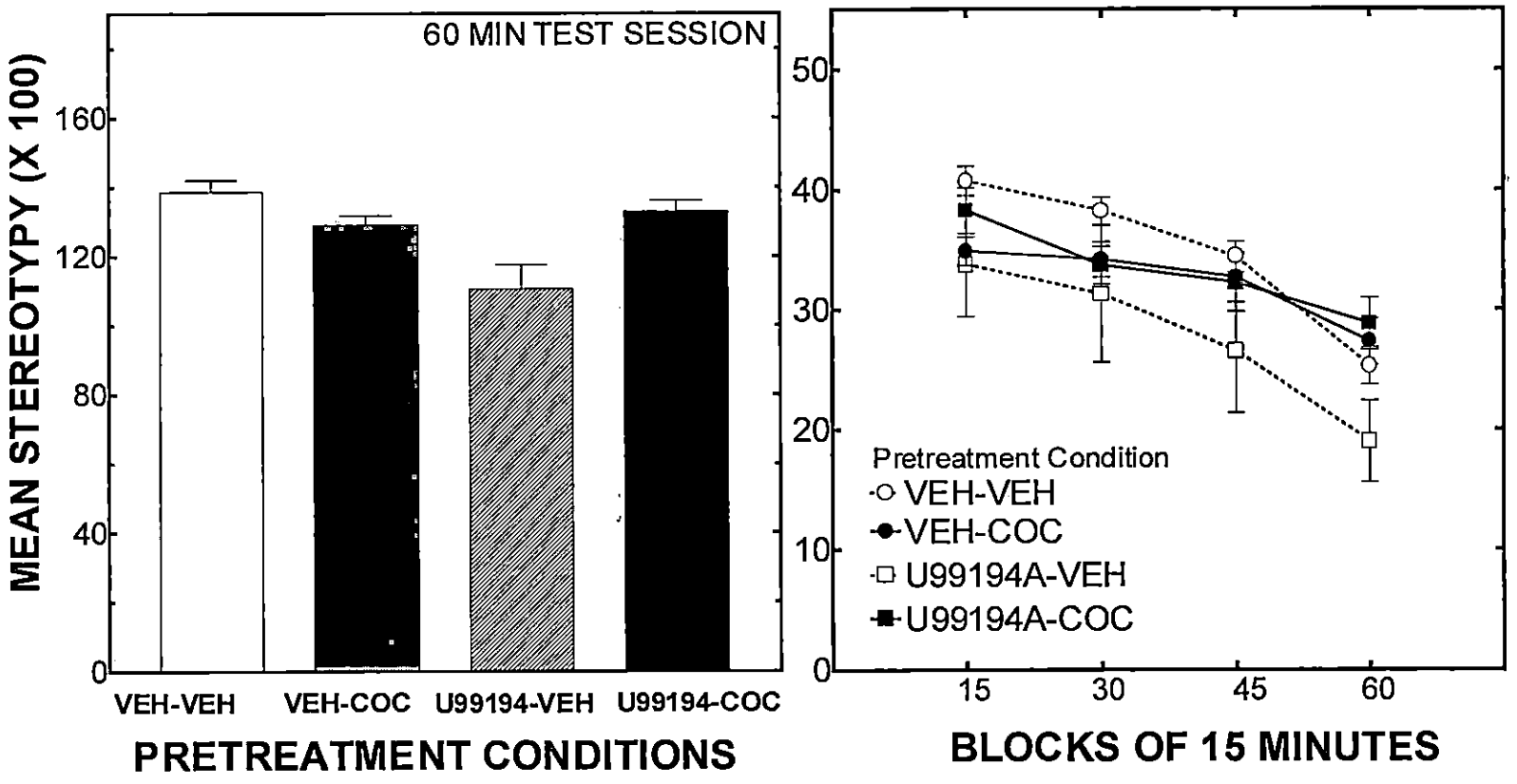

Figure 16. Mean stereotypic counts (± SEM) after a cocaine challenge injection (session 8) for rats previously treated chronically with either vehicle (VEH) or cocaine (15 mg/kg COC) in combination with either vehicle (VEH) or U99194A (1.00 mg/kg). The left panel represents the total session activity and the right panel presents the same data as a function of four 15 min blocks within the session

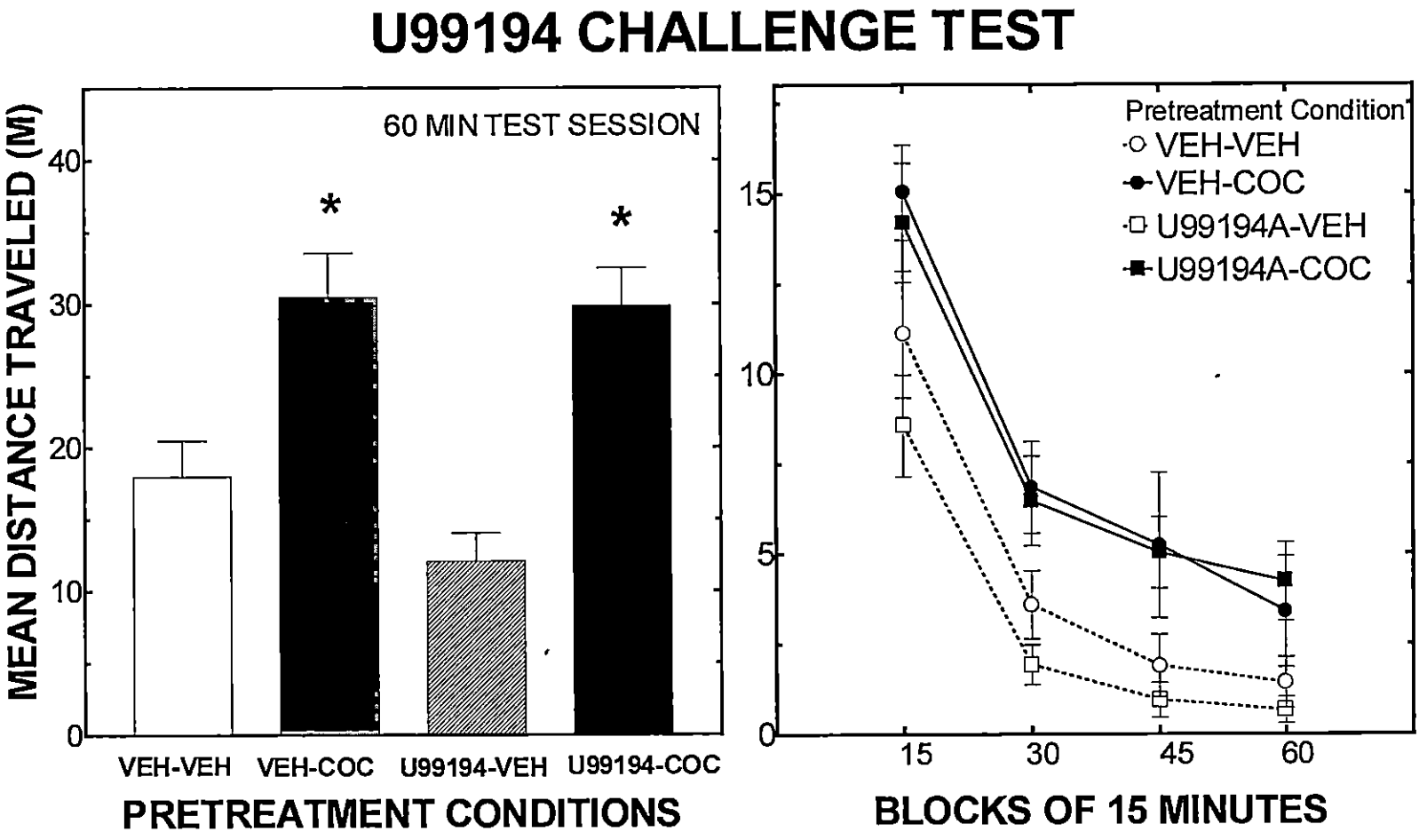

Figure 17. Mean distance traveled (± SEM) after a U99194A challenge injection (session 9) for rats previously treated chronically with either vehicle (VEH) or cocaine (15 mg/kg COC) in combination with either vehicle (VEH) or U99194A (1.00 mg/kg). The left panel represents the total session activity and the right panel presents the same data as a function of four 15 min blocks within the session

effects of the U99194A challenge injection, cocaine effect: $F(1,28) = 14.33$, $p < .001$. More important, prior treatments with U99194A alone did not significantly increase subsequent sensitivity to U99194A, and did not affect the influence of prior cocaine treatments, U99194A effect: $F(1,28) < 1.00$; U99194A x Cocaine interaction: $F < 1.00$.

Rears:

Figure 18 displays the mean number of rears for the four treatment groups after 1.0 mg/kg dose of U99194A. A summary of the ANOVA performed on this data is presented in Table 18. As may be seen in the left panel, pretreatment with cocaine significantly increased subsequent sensitivity to a challenge injection of U99194A, cocaine effect: $F(1,28) = 11.65$, $p < .01$, and this increase was not affected by concurrent U99194A pretreatment, U99194A effect: $F(1,28) < 1.00$, U99194A x cocaine interaction: $F(1,28) = 1.13$, $p = 0.296$. However, as shown on the right panel, all groups decreased activity across blocks, block effect: $F(3,84) = 107.29$, $p < .001$, but there were no interactions between U99194A and block factors, U99194A x Block interaction: $F(3,84) = 1.04$, $p = 0.378$; Cocaine x Block interaction: $F(3,84) = 11.65$, $p = .002$; U99194A x Cocaine x Block interaction: $F(3,84) < 1.00$].

Stereotypy:

Figure 19 displays the mean number of stereotypic counts for the four pretreatment groups after a 1.0mg/kg dose of U99194A. A summary of the ANOVA performed on this data is presented in Table 19. As may be seen in the left panel, pretreatment with cocaine increased the subsequent sensitivity to a challenge injection of U99194A, but pretreatment with 1.0 mg/kg U99194A did not significantly affect subsequent sensitivity to a challenge injection of U99194A, U99194A effect: $F(1,28) < 1.00$, cocaine effect: $F(1,28) = 15.56$, $p < .001$. Pretreatment with U99194A did

not affect the stereotypic counts of rats, U99194A x Cocaine interaction: $F(1,28)<1.00$. Stereotypic counts of all groups decreased across the blocks, block effect: $F(3,84) = 98.82$, $p<.001$. There were no interactions between U99194A and block, cocaine and block, U99194A, U99194A and cocaine factors, U99194A x Block interaction: $F(3,84) < 1.00$; Cocaine x Block interaction: $F(3,48) = 1.63$, $p=0.189$; U99194A x Cocaine x Block interaction: $F(3,84) < 1.00$.

U99194 CHALLENGE TEST

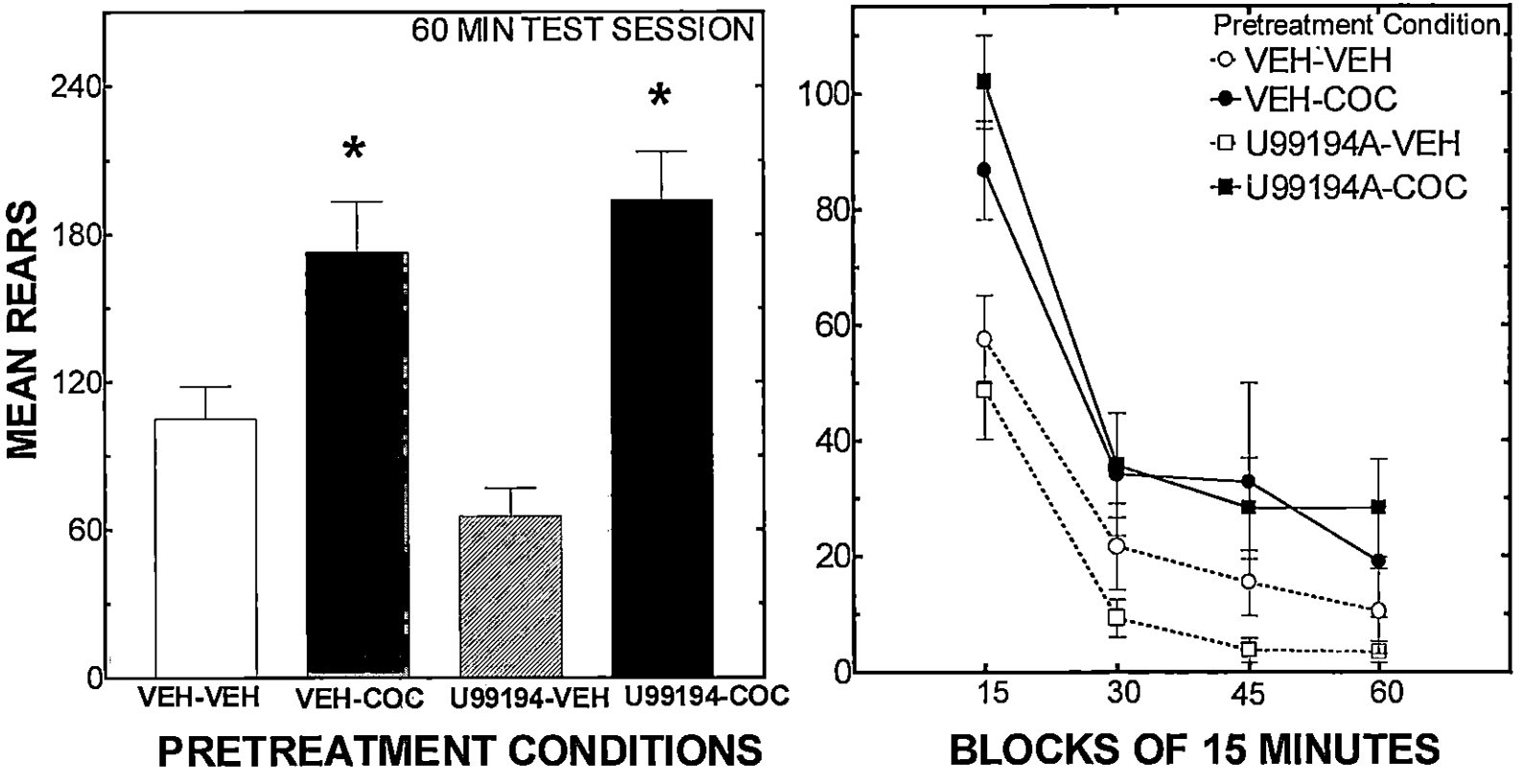

Figure 18. Mean number of rears (± SEM) after a U99194A challenge injection (session 9) for rats previously treated chronically with either vehicle (VEH) or cocaine (15 mg/kg COC) in combination with either vehicle (VEH) or U99194A (1.00 mg/kg). The left panel represents the total session activity and the right panel presents the same data as a function of four 15 min blocks within the session

U99194 CHALLENGE TEST

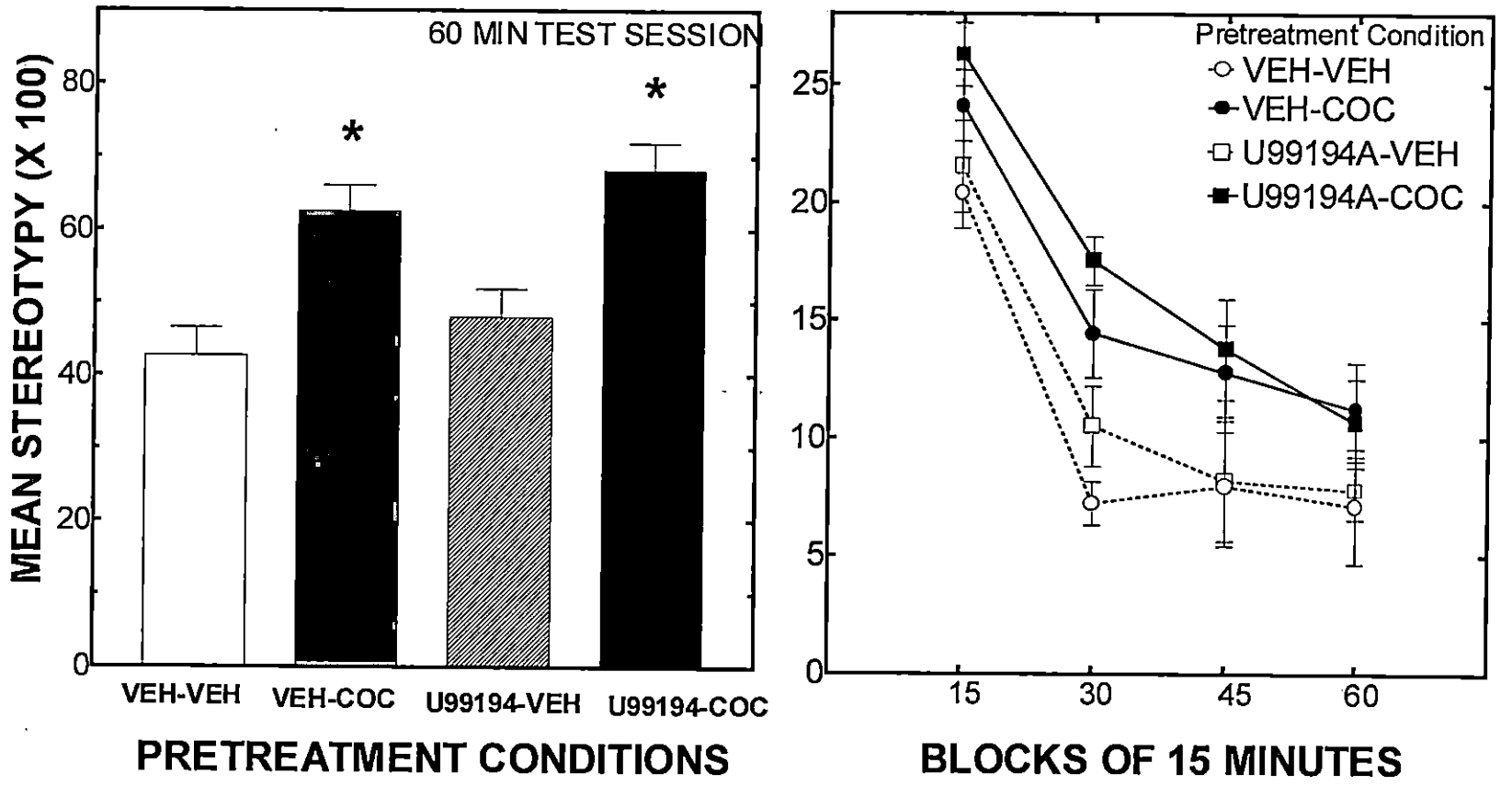

Figure 19. Mean stereotypic counts (± SEM) after a U99194A challenge injection (session 9) for rats previously treated chronically with either vehicle (VEH) or cocaine (15 mg/kg COC) in combination with either vehicle (VEH) or U99194A (1.00 mg/kg). The left panel represents the total session activity and the right panel presents the same data as a function of four 15 min blocks within the session

CHAPTER 5

DISCUSSION

Consistent with previous research, with repeated daily treatment, cocaine produced a greater increase in both horizontal (distance traveled) and vertical (rearing) activity, as well as stereotypic small movements compared to vehicle injections (e.g. Kalivas et al., 1988; Martin-Iverson & Reimer, 1994). Furthermore, repeated treatment with cocaine alone induced behavioral sensitization to horizontal locomotor-activating effects of cocaine, but not to rearing or stereotypic activity. This progressive increase in cocaine-induced locomotor activity is consistent with the sensitization effect previously reported for cocaine (e.g. Kalivas et al. 1988; Steketee, Striplin, Murray, & Kalivas, 1991).

In contrast to the cocaine-induced hyperactivity effect, U99194A treatments with both 0.1mg/kg and 1.0mg/kg did not significantly affect horizontal locomotion, stereotypic activity or rearing. However, in a preliminary experiment in our laboratory, U99194A did produce a small, but significantly increase in activity when administrated five minutes before activity testing (Mattingly, Rice, Langfels, & Fields, 2000). Similar results were reported by other studies (Waters, Svensson, Hassdsma, Smith, & Carlsson, 1993; Gendreau, Petitto, Schnauss, Frantz, & Hartesveldt, 1997). U99194A increased locomotor behavior over a wide dose range in Water's study (7-55mg/kg) and in Gendreau's studies (5-30mg/kg). This discrepancy between the current study and these previous works is probably related to methodological differences. For example, in our previous study (Mattingly et al., 2000), U99194A was administrated 5 min before activity testing. In contrast, U99194A was administrated 15 min before activity testing in the current experiments. Although U99194A did not significantly

increase activity in the present study, U99194A treatments did not inhibit locomotor activity. This finding contrast with previous research with dopamine D1- and D2- type antagonists which significantly suppress activity (Mattingly, et al.,1993,1994). The current finding, therefore, is consistent with the view that the functional role of D3 receptor differs from that of dopamine D1- and D2 receptors (Mattingly, et al. 1996, 1998). If U99194A affected D2- receptors then activity should have been decreased.

In contrast to D1 and D2 dopamine antagonists, U99194A did not significantly affect the acute cocaine-induced increase in locomotor activity. This result contrasts with the effects of more conventional dopamine D1-type (SCH23390) and D2-type (sulpiride, eticlopride et al.) receptor antagonists which decreased motor behavior over a wide dose range (Mattingly et al. 1993; Mattingly et al. 1996; Ferrari & Guiliani, 1995). Consistent with this finding, repeated treatment with U99194A enhanced, rather than inhibited the locomotor activity effect of amphetamine (Waters et al., 1993). Moreover, the dopamine D3-preferring receptor agonists PD128907 and quinpirole have been reported to attenuate, rather than enhance, the locomotor activity effects of amphetamine (Deboer, Enrico, Wright, Wise, & Timmerman, 1997; Defonseca, Rubio, Calderyon, & Coob, 1995). Similarly, the selective D3 receptor agonist 7-OH-DPAT attenuates the stimulating effects of cocaine (Mattingly et al., In press). Taken together, these findings suggest that dopamine D3 receptors, unlike D1- and D2-type receptors, may be inhibitory with respect to behavior (Waters et al., 1993).

Based on the previous research, we hypothesized that stimulation of dopamine D3 receptors may be critical to the development of behavioral sensitization to cocaine. This hypothesis was based primarily on the finding that concurrent treatments of a low dose of the selective dopamine D3 receptor agonist, 7-OH-DPAT with cocaine

enhances the development of behavioral sensitization to cocaine (Mattingly et al., in press). Based upon this finding, it was predicted that blocking dopamine D3 receptor with U99194A may prevent the development of behavioral sensitization to cocaine. This hypothesis was not supported. Neither dose of U99194A affected the development of behavioral sensitization to cocaine as measured across sessions or on the cocaine challenge test. This finding suggests that the stimulation of dopamine D3 receptors is not critical to the development of cocaine-induced behavioral sensitization. This finding is consistent with previous research using dopamine D2-type receptor antagonists (Mattingly et al., 1994; Wolf, White, Nasser,Brooderson & Khansa, 1993), and suggests that stimulation of individual dopamine receptors is not necessary for the development of behavioral sensitization. Moreover, these results are consistent with the findings that although 7-OH-DPAT partially substitutes for cocaine in drug discrimination tests, U99194A does not block the 7-OH-DPAT substitution for cocaine (Baker, Svensson, & Garner, 1998). Thus, like behavioral sensitization, D3 receptor may not be important for the subjective effects of cocaine.

Although repeated treatment with U99194A did not affect the development of behavioral sensitization to cocaine, chronic pretreatment with cocaine increased the activity response to the U99194A challenge injection. This finding suggests that dopamine D3 receptor function may be altered by chronic cocaine treatment. This result is consistent with the finding that dopamine D3 binding is significantly increased in human cocaine overdose victims (Staly & Mash, 1996). Moreover, this finding appears consistent with the view that postsynaptic D3 receptor may be inhibitory with respect to locomotor behavior (Svensson et al 1993; Waters et al., 1993). If so, then the increased activity observed in cocaine-pretreated rats may represent disinhibition.

Unfortunately, the validity of this interpretation cannot be determined with the present experimented design. That is, since cocaine-pretreated rats often display conditioned hyperactivity after vehicle injections (Durazzo, Gauvin, Goulden, & Briscoe, 1992; Nomikos & Spyraki,1988), the apparent U99194A-induced increase in activity may simply be due to conditioning response. It might be noted, however, that conditioning effects usually dissipate very quickly after the vehicle injection. In contrast, the hyperactivity in cocaine-pretreated rats after the U99194A challenge injection was maintained across the four fifteen minute blocks. This finding suggests that the hyperactivity observed may not be due to conditioning effects. However, additional research is needed to clarify the interpretation of this finding

In summary, the present results clearly demonstrate that concurrent treatment with U99194A and cocaine does not prevent the development of behavioral sensitization to cocaine. This finding suggests that stimulation of dopamine D3 receptor is not critical to the development of behavioral sensitization to cocaine. However, although U99194A treatments did not block cocaine-induced behavioral sensitization, chronic cocaine treatments altered the responsiveness of rats to a U99194A challenge injection. This finding tentatively suggests that dopamine D3 receptor function may be altered by chronic cocaine treatments. Currently, the validity of this interpretation is unclear. In conclusion, to the extent that the development of behavioral sensitization is a valid model for the development of cocaine craving, then the present results along with previous findings questioned the importance of dopamine D3 receptor as a critical component in cocaine craving.

CHAPTER 6

REFERENCES

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APPENDIX A
ANOVA SUMMARY TABLES

Table 2

Summary of Analysis of Variance Performed on Mean Distance Traveled during the Pretreatment Session in Experiment 1.

Source	Df	MS	F	p
Between Groups				
U99194A (U)	1	7.864 E+06	2.23	0.1467
Cocaine (C)	1	7.443 E+08	210.91	0.0001**
U x C	1	5.486 E+06	1.55	0.2228
Error	28	9.882 E+07		
Within Groups				
Session (S)	6	7.809 E + 06	8.92	0.0001**
U x S	6	8.164 E + 05	0.93	0.4728
C x S	6	1.445 E + 07	16.52	0.0001**
U x C x S	6	7.416 E + 05	0.85	0.5350
Error	168	1.470 E + 08	0.88	
Block (B)	3	9.558 E + 07	336.06	0.0001**
U x B	3	3.943 E + 05	1.39	0.2526
C x B	3	3.610 E + 07	126.94	0.0001**
U x C x B	3	2.239 E + 05	0.79	0.5044
Error	84	4.937 E + 07		
S x B	18	3.892 E + 05	3.97	0.0001**
U x S x B	18	6.982 E + 04	0.71	0.7993
C x S x B	18	8.689 E + 05	8.87	0.0001**
U x C x S x B	18	8.179 E + 04	0.84	0.6589
Error	504	4.937 E + 07		

Table 3

Summary of Analysis of Variance Performed on Mean Number of Rears during the Pretreatment Session in Experiment 1

Source	df	MS	F	p
Between Groups				
U99194A (U)	1	1.479 E +02	0.01	0.9143
Cocaine (C)	1	6.534 E +05	52.10	0.0001**
U x C	1	6.480 E +02	0.05	0.8218
Error	28	1.254 E +04		
Within Groups				
Session (S)	6	9.026 E +03	4.48	0.0003**
U x S	6	9.385 E +02	0.47	0.8328
C x S	6	2.081 E +03	0.05	0.4054
U x C x S	6	7.108 E +02	0.35	0.9074
Error	168	2.014 E +03		
Block (B)	3	2.077 E +05	164.31	0.0001**
U x B	3	1.348 E +05	0.11	0.9560
C x B	3	1.141 E +04	9.02	0.0001**
U x C x B	3	1.437 E +03	1.14	0.3389
Error	84	1.264 E +03		
S x B	18	2.220 E +03	4.88	0.0001**
U x S x B	18	1.872 E +02	0.41	0.9856
C x S x B	18	9.564 E +02	2.10	0.0052**
U x C x S x B	18	2.443 E +03	0.54	0.9407
Error	504	4.552 E +02		

Table 4

Summary of Analysis of Variance Performed on Mean Stereotypic Counts during the Pretreatment Session in Experiment 1

Source	df	MS	F	p
Between Groups				
U99194A (U)	1	4.812 E + 05	0.25	0.6224
Cocaine (C)	1	8.668 E + 08	446.71	0.0001**
U x C	1	3.370 E + 04	0.02	0.8961
Error	28	1.940 E + 06		
Within Groups				
Session (S)	6	1.570 E + 06	1.89	0.0858
U x S	6	1.631 E + 06	2.03	0.0640
C x S	6	7.397 E + 06	8.89	0.0001**
U x C x S	6	1.083 E + 06	1.30	0.2591
Error	168	8.323 E + 05		
Block (B)	3	9.595 E + 07	314.55	0.0001**
U x B	3	2.980 E + 05	0.98	0.4076
C x B	3	5.867 E + 06	19.23	0.0001**
U x C x B	3	7.680 E + 05	2.52	0.0636
Error	84	3.050 E + 05		
S x B	18	5.035 E + 05	3.60	0.0001**
U x S x B	18	7.147 E + 04	0.51	0.9535
C x S x B	18	2.782 E + 05	1.99	0.0091**
U x C x S x B	18	9.448 E + 04	0.68	0.8368
Error	504	1.399 E + 05		

Table 5

Summary of Analysis of Variance Performed on Mean Distance Traveled on the Cocaine (15mg/kg) Challenge Day of Experiment 1.

Source	df	MS	F	p
Between Groups				
U99194A (U)	1	2.827 E +04	0.01	0.9099
Cocaine (C)	1	4.725 E +07	21.79	0.0001**
U x C	1	7.001 E +05	0.32	0.5744
Error	28	6.071 E +07		
Within Groups				
Blocks (B)	3	2.871 E +07	149.05	0.0001**
U x B	3	1.085 E +05	0.56	0.6406
C x B	3	1.898 E +06	9.86	0.0001**
U x C x B	3	1.699 E +04	0.09	0.9663
Error	84			

Table 6

Summary of Analysis of Variance Performed on Mean Number of Rears on the Cocaine (15mg/kg) Challenge Day of Experiment 1.

Source	df	MS	F	p
Between Groups				
U99194A (U)	1	2.008 E +03	0.31	0.5850
Cocaine (C)	1	3.413 E +03	0.52	0.4774
U x C	1	4.430 E +03	0.67	0.4189
Error	28	6.581 E +3		
Within Groups				
Blocks (B)	3	2.314 E +02	25.70	0.0001**
U x B	3	5.622 E +02	0.62	0.6011
C x B	3	1.497 E +02	0.17	0.9189
U x C x B	3	6.563 E +02	0.73	0.5374
Error	84	9.003 E +02		

Table 7

Summary of Analysis of Variance Performed on Mean Stereotypic Counts on the Cocaine (15mg/kg) Challenge Day of Experiment 1.

Source	df	MS	F	p
Between Groups				
U99194A (U)	1	6.808 E +04	0.06	0.8021
Cocaine (C)	1	1.808 E +06	1.70	0.2028
U x C	1	1.347 E +05	0.13	0.7246
Error	28	1.063 E +06		
Within Groups				
Blocks (B)	3	1.233 E +07	86.38	0.0001**
U x B	3	1.153 E +05	0.81	0.4931
C x B	3	4.030 E +05	2.82	0.0437*
U x C x B	3	2.572 E +05	1.80	0.1531
Error	84	1.427 E +05		

Table 8

Summary of Analysis of Variance Performed on Mean Distance Traveled on the U99194A (0.1mg/kg) Challenge Day in Experiment 1.

Source	df	MS	F	p
Between Groups				
U99194A (U)	1	9.262 E +04	0.62	0.4377
Cocaine (C)	1	2.918 E +06	19.53	0.0001**
U x C	1	1.458 E +04	0.10	0.7570
Error	28	1494 E +05		
Within Groups				
Blocks (B)	3	4.330 E +06	80.76	0.0001**
U x B	3	7.703 E +04	1.44	0.2379
C x B	3	3.979 E +05	7.42	0.0002**
U x C x B	3	2.227 E +04	0.42	0.7424
Error	84	5.362 E +04		

Table 9

Summary of Analysis of Variance Performed on Mean Number Rears on the U99194A (0.1mg/kg) Challenge Day in Experiment 1.

Source	df	MS	F	p
Between Groups				
U99194A (U)	1	8.778 E +01	0.13	0.7204
Cocaine (C)	1	8.256 E +03	12.30	0.0016**
U x C	1	6.125 E +02	0.91	0.3477
Error	28			
Within Groups				
Blocks (B)	3	1.161 E +04	44.11	0.0001**
U x B	3	1.116 E +02	0.42	0.7363
C x B	3	3.521 E +02	1.34	0.2676
U x C x B	3	1.122 E +02	0.43	0.7345
Error	84	2.631 E +02		

Table 10

Summary of Analysis of Variance Performed on Mean Stereotypic Counts on the U99194A (0.1mg/kg) Challenge Day in Experiment 1.

Source	df	MS	F	p
Between Groups				
U99194A (U)	1	6.010 E +05	1.15	0.2923
Cocaine (C)	1	8.048 E +06	15.43	0.0005**
U x C	1	7.459 E +02	0.00	0.9701
Error	28	5.217 E +05		
Within Groups				
Blocks (B)	3	1.270 E +07	65.44	0.0001**
U x B	3	1.513 E +05	0.78	0.5086
C x B	3	1.991 E +05	1.03	0.3855
U x C x B	3	2.181 E +04	0.11	0.9526
Error	84	1.941 E +05		

Table 11

Summary of Analysis of Variance Performed on Mean Distance Traveled During the Pretreatment Session in Experiment 2.

Source	Df	MS	F	p
Between Groups				
U99194A (U)	1	6.653 E+06	1.84	0.1850
Cocaine (C)	1	5.616 E+08	155.61	0.0001**
U x C	1	7.458 E+05	0.21	0.6529
Error	28	3.609 E+06		
Within Groups				
Session (S)	6	3.364 E + 06	5.12	0.0001**
U x S	6	3.142 E + 05	0.48	0.8242
C x S	6	6.786 E + 06	10.32	0.0001**
U x C x S	6	1.883E + 05	0.29	0.9428
Error	168	6.575 E + 05		
Block (B)	3	6.328 E + 07	177.56	0.0001**
U x B	3	7.354 E + 05	2.06	0.1112
C x B	3	1.492E + 07	41.87	0.0001**
U x C x B	3	2.239 E + 05	1.65	0.1851
Error	84	4.937 E + 07		
S x B	18	1.656 E + 05	2.47	0.0007*
U x S x B	18	5.642 E + 04	0.84	0.6509
C x S x B	18	3.434 E + 05	5.12	0.0001**
U x C x S x B	18	3.560 E + 04	0.53	0.9429
Error	504	6.704 E + 04		

Table 12

Summary of Analysis of Variance Performed on Mean Number of Rears during the Pretreatment Session in Experiment 2.

Source	df	MS	F	p
Between Groups				
U99194A (U)	1	7.018 E +02	0.12	0.7345
Cocaine (C)	1	1.165 E +06	194.85	0.0001**
U x C	1	6.103 E +04	10.21	0.0034**
Error	28	5979 E +03		
Within Groups				
Session (S)	6	1.340 E +04	3.06	0.0073**
U x S	6	2.134 E +03	0.49	0.8177
C x S	6	5.445 E +03	1.24	0.2873
U x C x S	6	2.662 E +03	0.61	0.7244
Error	168	4.384 E +03		
Block (B)	3	2.507 E +05	183.04	0.0001**
U x B	3	1.341 E +02	0.10	0.9610
C x B	3	2.505 E +04	18.29	0.0001**
U x C x B	3	6.572 E +03	0.48	0.6972
Error	84	1.369 E +03		
S x B	18	1.935 E +03	3.92	0.0001**
U x S x B	18	5.534 E +02	1.12	0.3263
C x S x B	18	1.032 E +03	2.09	0.0054**
U x C x S x B	18	1.382 E +02	0.28	0.9987
Error	504	4.932 E +02		

Table 13

Summary of Analysis of Variance Performed on Mean Stereotypic Counts During the Pretreatment Session in Experiment 2.

Source	df	MS	F	p
Between Groups				
U99194A (U)	1	4.527 E + 06	2.32	0.1388
Cocaine (C)	1	9.952 E + 08	510.35	0.0001**
U x C	1	7.591 E + 05	0.39	0.5377
Error	28	1.950 E + 06		
Within Groups				
Session (S)	6	2.339 E + 06	3.45	0.0031**
U x S	6	1.519 E + 05	0.22	0.9685
C x S	6	3.811 E + 06	5.62	0.0001**
U x C x S	6	3.137 E + 05	0.46	0.8351
Error	168	6.780 E + 05		
Block (B)	3	8.337 E + 07	237.16	0.0001**
U x B	3	6.229 E + 05	1.77	0.1587
C x B	3	5.248 E + 06	14.93	0.0001**
U x C x B	3	5.700 E + 05	0.16	0.9215
Error	84	3.515E + 05		
S x B	18	6.878 E + 05	6.88	0.0001**
U x S x B	18	8.833 E + 04	0.88	0.5991
C x S x B	18	1.697 E + 05	1.70	0.0362*
U x C x S x B	18	1.007 E + 05	1.01	0.4490
Error	504	9.996 E + 04		

Table 14

Summary of Analysis of Variance Performed on Mean Distance Traveled on the Cocaine (15mg/kg) Challenge Day in Experiment 2.

Source	df	MS	F	p
Between Groups				
U99194A (U)	1	1.081 E +06	0.52	0.4789
Cocaine (C)	1	1.164 E +08	55.44	0.0001**
U x C	1	2.067 E +03	0.00	0.9572
Error	28	2.099 E +06		
Within Groups				
Blocks (B)	3	9.918 E +06	76.92	0.0001**
U x B	3	9.974E +04	0.77	0.5120
C x B	3	4.007E +06	31.08	0.0001**
U x C x B	3	6.206 E +04	0.48	0.6962
Error	84	1.289E +05		

Table 15

Summary of Analysis of Variance Performed on Mean Number of Rears on the Cocaine (15mg/kg) Challenge Day in Experiment 2.

Source	df	MS	F	p
Between Groups				
U99194A (U)	1	1.922 E +03	0.22	0.6444
Cocaine (C)	1	5.806 E +04	6.58	0.0160*
U x C	1	1.320 E +02	0.01	0.9035
Error	28	8.827 E +03		
Within Groups				
Blocks (B)	3	5.111 E +04	33.15	0.0001**
U x B	3	7.300 E +02	0.47	0.7016
C x B	3	1.524 E +03	0.99	0.4022
U x C x B	3	5.238 E +03	3.40	0.0215*
Error	84	1.542 E +03		

Table 16

Summary of Analysis of Variance Performed on Mean Stereotypic Counts on the Cocaine (15mg/kg) Challenge Day in Experiment 2.

Source	df	MS	F	p
Between Groups				
U99194A (U)	1	2.903 E +06	1.36	0.2528
Cocaine (C)	1	8.187 E +05	0.38	0.5402
U x C	1	5.129 E +06	2.41	0.1319
Error	28	2.130 E +06		
Within Groups				
Blocks (B)	3	8.331 E +06	68.43	0.0001**
U x B	3	9.823 E +05	0.81	0.4935
C x B	3	7.945 E +05	6.53	0.0005**
U x C x B	3	5.505 E +05	0.45	0.7164
Error	84	1.217 E +05		

Table 17

Summary of Analysis of Variance Performed on Mean Distance Traveled on the U99194A (1.0mg/kg) Challenge Day in Experiment 2.

Source	df	MS	F	p
Between Groups				
U99194A (U)	1	2.105 E +05	0.66	0.4248
Cocaine (C)	1	4.597 E +06	14.33	0.0007**
U x C	1	1.414 E +05	0.44	0.5122
Error	28	3.209 E +05		
Within Groups				
Blocks (B)	3	6.412 E +06	119.10	0.0001**
U x B	3	4.207 E +04	0.78	0.5076
C x B	3	5.511 E +04	1.02	0.3864
U x C x B	3	3.746 E +03	0.07	0.9760
Error	84	5.383 E +04		

Table 18

Summary of Analysis of Variance Performed on Mean Number Rears on the U99194A (1.0mg/kg) Challenge Day in Experiment 2.

Source	df	MS	F	p
Between Groups				
U99194A (U)	1	1.665 E +02	0.10	0.7535
Cocaine (C)	1	1.931 E +04	11.65	0.0020**
U x C	1	1.876 E +03	1.13	0.2964
Error	28	1.657 E +03		
Within Groups				
Blocks (B)	3	2.349 E +04	107.29	0.0001**
U x B	3	2.282 E +02	1.04	0.3781
C x B	3	1.016 E +03	4.64	0.0047**
U x C x B	3	9.697 E +01	0.44	0.7229
Error	84	2.189 E +02		

Table 19

Summary of Analysis of Variance Performed on Mean Stereotypic Counts on the U99194A (1.0mg/kg) Challenge Day in Experiment 2.

Source	df	MS	F	p
Between Groups				
U99194A (U)	1	2.938 E +05	0.27	0.6081
Cocaine (C)	1	1.699 E +07	15.56	0.0005**
U x C	1	1.026 E +06	0.94	0.3407
Error	28	1.092 E +06		
Within Groups				
Blocks (B)	3	2.035 E +07	98.82	0.0001**
U x B	3	6.273 E +04	0.30	0.8219
C x B	3	3.348 E +05	1.63	0.1894
U x C x B	3	1.751 E +04	0.09	0.9680
Error	84	2.059 E +05		

APPENDIX B

COUNTERBALANCING

Squad#	Subject#	Pretreatment Group	Chamber
1	1	Vehicle-Vehicle	1
1	2	Vehicle-Cocaine	2
1	3	U99194A-Vehicle	3
1	4	U99194A-Cocaine	4
2	5	Vehicle-Cocaine	1
2	6	Vehicle-Vehicle	2
2	7	U99194A-Cocaine	3
2	8	U99194A-Vehicle	4
3	9	U99194A-Vehicle	1
3	10	U99194A-Cocaine	2
3	11	Vehicle-Vehicle	3
3	12	Vehicle-Cocaine	4
4	13	U99194A-Cocaine	1
4	14	U99194A-Vehicle	2
4	15	Vehicle-Cocaine	3
4	16	Vehicle-Vehicle	4
5	17	Vehicle-Vehicle	1
5	18	Vehicle-Cocaine	2
5	19	U99194A-Vehicle	3
5	20	U99194A-Cocaine	4
6	21	Vehicle-Cocaine	1
6	22	Vehicle-Vehicle	2
6	23	U99194A-Cocaine	3
6	24	U99194A-Vehicle	4
7	25	U99194A-Vehicle	1
7	26	U99194A-Cocaine	2
7	27	Vehicle-Vehicle	3
7	28	Vehicle-Cocaine	4
8	29	U99194A-Cocaine	1
8	30	U99194A-Vehicle	2
8	31	Vehicle-Cocaine	3
8	32	Vehicle-Vehicle	4